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Enantiomeric profiling of chiral illicit drugs in a pan-European study

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Abstract

The aim of this paper is to present the first study on spatial and temporal variation in the enantiomeric profile of chiral drugs in eight European cities. Wastewater-based epidemiology (WBE) and enantioselective analysis were combined to evaluate trends in illicit drug use in the context of their consumption vs direct disposal as well as their synthetic production routes. Spatial variations in amphetamine loads were observed with higher use in Northern European cities. Enantioselective analysis showed a general enrichment of amphetamine with the *R*-(-)-enantiomer in wastewater indicating its abuse. High loads of racemic methamphetamine were detected in Oslo (EF = 0.49 ± 0.02). This is in contrast to other European cities where *S*-(+)-methamphetamine was the predominant enantiomer. This indicates different methods of methamphetamine synthesis and/or trafficking routes in Oslo, compared with the other cities tested. An enrichment of MDMA with the *R*-(-)-enantiomer was observed in European

wastewaters indicating MDMA consumption rather than disposal of unused drug. MDA's chiral signature indicated its enrichment with the *S*-(+)-enantiomer, which confirms its origin from MDMA metabolism in humans. HMMA was also detected at quantifiable concentrations in wastewater and was found to be a suitable biomarker for MDMA consumption. Mephedrone was only detected in wastewater from the United Kingdom with population-normalised loads up to 47.7 mg 1000 people^{-1} day^{-1}. The enrichment of mephedrone in the *R*-(+)-enantiomer in wastewater suggests stereoselective metabolism in humans, hence consumption, rather than direct disposal of the drug. The investigation of drug precursors, such as ephedrine, showed that their presence was reasonably ascribed to their medical use.

Keywords

Wastewater-based epidemiology; Illicit Drugs; Chiral Drugs; Enantioselective analysis.

1. Introduction

Since the first study by Zuccato et al. (Zuccato, Chiabrando et al. 2005), where wastewater-based epidemiology (WBE) was introduced as an approach to estimate community-wide illicit drug use trends, WBE has proven to provide valuable and complementary information to traditional epidemiological approaches (Thomas and Reid 2011, Kasprzyk-Hordern, Bijlsma et al. 2014). Indeed, the analysis of carefully selected biomarkers, which are often unique human urinary metabolic excretion products, has allowed for near real-time profiling of the community-wide use of a number of illicit drugs (Thomas, Bijlsma et al. 2012, Ort, van Nuijs et al. 2014), new psychoactive substances (NPS) (Reid, Derry et al. 2014, Castiglioni, Borsotti et al. 2015), alcohol (Reid, Langford et al. 2011) and tobacco (Castiglioni, Senta et al. 2014) use and counterfeit medicines (Causanilles, Emke et al. 2016). The study by Zuccato et al. was followed and further developed by other research groups (Van Nuijs, Pecceu et al. 2009, van Nuijs, Pecceu et al. 2009, Karolak, Nefau et al. 2010, Metcalfe, Tindale et al. 2010, Terzic, Senta et al. 2010, Reid, Langford et al. 2011, van Nuijs, Castiglioni et al. 2011). The first Europe-wide study in 2011, led by the SCORE group (www.score-cost.eu), involved 19 cities and estimated temporal and spatial drugs use trends across Europe (Thomas, Bijlsma et al. 2012). This was followed by Europe-wide monitoring of 23 cities in 2012 (Ort, van Nuijs et al. 2014) and then 42 cities in 2013 (http://www.emcdda.europa.eu/topics/pods/waste-water-analysis 2016). WBE is currently used to report on world-wide illicit drug use trends (Lai, O'Brien et al. 2016, Tscharke, Chen et al. 2016) and feeds into the Europe-wide evidence based early warning system managed by the European Monitoring Centre for Drugs & Drug Addiction (EMCDDA) (http://www.emcdda.europa.eu/activities/wastewater-analysis).

There are several key stages that need to be considered when developing new WBE applications: (i) biomarker selection; (ii) collection of representative wastewater samples; (iii) measurement of biomarkers in wastewater; (iv) calculation of population-normalised mass loads and, finally, (v) estimation of the consumption *pro capita*. Biomarker selection is considered to be of critical importance. This cannot be limited to the parent drug itself if the determination of drug consumption estimate is the aim, since bias related to disposal of the

unused drug might take place. A biomarker should be uniquely formed in the body, be stable and present in wastewater at quantifiable concentrations. Furthermore, the impact of transformation of biomarkers in sewer biofilm/suspended solids between the discharge and the sampling points should be considered as it could affect the detected amount of the analytes, thereby influencing epidemiological observations (McCall, Scheidegger et al. 2016, Ramin, Libonati Brock et al. 2016). Unfortunately, as it is not always possible to select a unique metabolic biomarker, different solutions need to be sought. One of the innovative approaches focuses on enantiomerism of chiral drugs and their stereoselective human metabolism [26].

Enantiomeric profiling can complement WBE data with valuable information on abuse trends and potency of chiral drugs. It can also help with distinguishing between the legal and illicit use of drugs, as well as providing an indication of actual consumption as opposed to disposal of non-consumed drugs [2]. This is because drug synthesis is associated with different chiral signatures that depend on the routes of synthesis. Furthermore, chiral drugs undergo stereoselective disposition in humans leading to changes in their chiral signature (expressed as enantiomeric fraction, EF) (Kasprzyk-Hordern 2010) when excreted.

The potential of enantioselective analysis for WBE purposes has thus far only been demonstrated in a few limited studies focussing on (i) verification of the fate of chiral drugs during wastewater treatment and in the environment (Camacho-Muñoz 2015), (ii) confirmation of origin of amphetamine found in wastewater in the United Kingdom (UK) (Kasprzyk-Hordern and Baker 2012) and (iii) confirmation of MDA present in wastewater as a result of MDMA consumption rather than MDA use (Kasprzyk-Hordern and Baker 2012). Vázquez-Roig et al. (Vazquez-Roig, Kasprzyk-Hordern et al. 2014) reported usage patterns of chiral drugs in the catchment area of Valencia (Spain), by linking selective enrichment of MDMA with the *R*-(-)-enantiomer in wastewater to human consumption. Enantioselective analysis also proved invaluable in establishing that the unexpectedly high quantity of MDMA detected during a monitoring campaign in 2011 in Utrecht was due to direct disposal of unused MDMA as a consequence of a police raid at a nearby illegal production facility (Emke, Evans et al. 2014) and not as a result of high levels of consumption. Similarly, Petrie et al. (Petrie, Youdan et al. 2016) linked high levels of fluoxetine in wastewater with the disposal of the unused drug rather than its consumption. Recently, Castrignanò et al. (Castrignanò, Lubben et al. 2016) found mephedrone enriched with R-(+)-enantiomer in wastewater in the UK suggesting human use.

Despite these findings, a limited number of studies have correlated the enantiomeric composition of chiral biomarkers to official statistics (Camacho-Muñoz 2015). Hence, this is the first pan-European study aimed at investigating enantiomeric profiling of “common” drugs of abuse, NPS and chiral drug precursors in eight cities from different countries with a total population equivalent of 4.9 million. The focus of this research was to:

- quantify selected drugs in wastewater from eight European cities,
- verify if drug residues in wastewater originated from the direct disposal of unused drugs into the sewer system or their consumption.

2. Experimental

2.1. Chemicals and materials

The following chiral analytes were selected in this study (Figure S1): (±)-mephedrone, (±)-4-hydroxy-3-methoxymethamphetamine (HMMA), (±)-3,4-methylenedioxymethamphetamine (MDMA), (±)-4-hydroxy-3-methoxyamphetamine (HMA), (±)-methamphetamine, (±)-amphetamine, (±)-3,4-methylenedioxyamphetamine (MDA), (±)-3,4-methylenedioxy-N-ethyl-amphetamine (MDEA), (±)-ephedrine, (±)-pseudoephedrine, (±)-para-methoxyamphetamine (PMA), (±)-norephedrine. Table S1 shows properties of all analytes. Amphetamine-D_5, methamphetamine-D_5, mephedrone-D_3, MDA-D_5, MDMA-D_5, MDEA-D_5 and *1S,2R*-(+)-ephedrine-D_3 were used as internal standards (ISs).

All standards and ISs were of the highest purity available (>97%). Stock and working solutions of standards were stored at -20 °C. Methanol, acetonitrile and ammonium acetate were purchased from Sigma Aldrich, UK. Ultrapure water was obtained from MilliQ system (UK). Deactivation of the glassware was carried out as described in (Castrignanò, Lubben et al. 2016) to prevent the adsorption of basic analytes to the hydroxyl sites on the glass surface.

2.2.Sample collection, storage and sample preparation

24-hour composite wastewater influent samples were collected over seven consecutive days in March 2015 from wastewater treatment plants (WWTPs) across Europe using best practice sampling protocol (Castiglioni, Thomas et al. 2014). The week in March was chosen as a "routine week", in which no national and local festivities were taking place. Sampling sites were in Norway (Oslo), United Kingdom (Bristol), Denmark (Copenhagen), The Netherlands (Utrecht), Belgium (Brussels), Switzerland (Zurich), Italy (Milan) and Spain (Castellón). Table S2 provides information on population and flow for the selected cities in the study. After collection, samples were transported to the local laboratory in refrigerated conditions and shipped on ice blocks to the UK within 24 hours. A fully validated analytical method was used for the detection and quantification of chiral drugs of abuse in wastewater as described elsewhere (Castrignanò, Lubben et al. 2016).

2.3.Sample analysis

Samples were analysed in triplicate using enantioselective high performance liquid chromatography coupled with tandem mass spectrometry system. Separation of all chiral analytes was undertaken with a CHIRALPAK® CBH HPLC column 5 µm particle size, L × I.D. 10 cm × 2.0 mm with a chiral-CBH guard column 10 × 2.0 mm, 5 µm particle size (Chiral Technologies, France) using a Waters ACQUITY UPLC® system (Waters, Manchester, UK) under isocratic conditions at a 0.1 mL min^{-1}. The mobile phase was a solution 1 mM ammonium acetate/methanol 85:15 v/v. The temperature was kept at 4 °C in the ACQUITY UPLC™ autosampler, whilst at 25 °C in the column compartment. The injection volume was set at 20 µL.

A triple quadrupole mass spectrometer (Xevo TQD, Waters, Manchester, UK) equipped with an electrospray ionisation source was used in positive mode operating in the multiple reaction monitoring (MRM) mode. Table S3 shows MRM transitions used for selected analytes. MassLynx 4.1 (Waters, UK) was used to control the Waters ACQUITY system and the Xevo TQD. Data processing was carried out using TargetLynx software (Waters, Manchester, UK). Method validation data are provided in Tables S4-S8.

2.4.Calculations

Enantiomeric fraction (EF) was calculated using the following equation (1):

$$EF = \frac{(+)}{[(+)+(-)]} \quad (1)$$

where (+) is the concentration of (+)-enantiomer or the first eluted enantiomer and (-) is the concentration of (-)-enantiomer or the second eluted enantiomer. EF equals 0.5 in the case of a racemate, whilst 1 or 0 in the case of the enantiopure compound.

In order to obtain daily mass loads, the concentrations of analytes expressed in ng L^{-1} (see Table S9) were multiplied by the flow rate (L day^{-1}) and then normalised by the population size of the catchment area. This was essential for comparing data coming from different cities involved in the study.

All relevant information on the selected chiral illicit drugs is gathered in Table S10. It includes: biomarkers used as drug target residue (DTR), urinary excretion data, correction factors (CFs) used for WBE estimates, EF expected in urine after human metabolism (EF_{urine}), EF calculated from illegal synthesis of the drug ($EF_{illegal\_synth}$), information derived from the legal use of the drug with EF derived from the legal use of the drug ($EF_{legal\ source}$) and consumption estimates from official health statistics and from wastewater analysis. CF was calculated as the ratio between the molar ratio of the drug and its DTR and the urinary excretion data.

Estimated community-wide consumptions were calculated using population-normalised mass loads and CF.

3. Results and Discussion

3.1.Amphetamines

Data on amphetamines consumption, reported by the European drug report 2015 (as a sum of amphetamine and methamphetamine), showed that 1.3 million Europeans within the ages of 15 - 34 used amphetamines in the last year (EMCDDA 2015). This data was obtained using the EMCDDA's five key epidemiological indicators, which consist of "estimates of recreational use (based mainly on surveys), estimates of high-risk use, drug-related deaths, infectious diseases and drug treatment entry" along with Reitox focal points and other sources (EMCDDA 2015). In this work, we applied WBE to estimate amphetamine and methamphetamine use in eight European cities. Unfortunately, no metabolic biomarkers of amphetamine and methamphetamine are validated for a reliable estimation of their abuse via WBE. Therefore, amphetamine and methamphetamine themselves are commonly used as biomarkers. This constitutes a problem since the analysis of parent drugs does not allow for distinguishing between consumed and unconsumed (meth)amphetamine. Additionally, amphetamine is also a metabolite of other (prescription) drugs, such as fenethylline, fenproporex, methamphetamine (Baselt) and selegiline (Ort, van Nuijs et al. 2014). Furthermore, the percentage of the unchanged amphetamine fraction in urine can change due to changes in urine pH (Table S10), leading to high uncertainty of calculations and possible over or underestimation of

amphetamine use. The awareness of this uncertainty is well recognised in the scientific community studying amphetamine use using WBE (Chiaia-Hernandez, Banta-Green et al. 2011), (Kasprzyk-Hordern, Dinsdale et al. 2009), (Postigo, Lopez de Alda et al. 2010), (van Nuijs, Mougel et al. 2011). As reported by Ort et al. (Ort, van Nuijs et al. 2014), the estimation of the amphetamine consumption has to be carried out in the context of methamphetamine data to distinguish between drug consumption from its metabolism. However, verification of the amphetamine/methamphetamine ratio cannot provide comprehensive information on drug consumption against direct disposal of unused drug. Additional evidence is therefore needed to distinguish between amphetamine abuse from its direct disposal or its usage as a prescription drug. The phenomenon of enantiomerism of amphetamines may provide invaluable insight (see section S1-2 for further information).

3.1.1. Amphetamine

Population-normalised amphetamine loads were <5 mg day^{-1} 1000 people^{-1} in Milan to a maximum weekly average value of 122.3 mg day^{-1} 1000 people^{-1} in Oslo, which shows higher amphetamine prevalence in Northern Europe (Figures 1a and S2, estimated consumptions also shown in Table S11). There was a decreasing amphetamine usage from Northern to Southern cities with only Italian and Spanish cities notably below the overall mean load of 28 mg day^{-1} 1000 people^{-1} reported in the 2013 European study (Ort, van Nuijs et al. 2014). By looking at the results from previous monitoring studies undertaken since 2012 (Ort, van Nuijs et al. 2014), temporal trends show that amphetamine loads increased in Oslo, Copenhagen, Brussels and Milan, even if they are very low for the latter city. They remained stable in Bristol and decreased in Zurich and in Utrecht.

Enantiomeric profiling revealed that amphetamine in wastewater was enriched with the *R*-(-)-enantiomer in most European cities (EF_{ww}<0.5, EF determined in the wastewater is referred as EF_{ww}; the enrichment was significant as the unpaired t-test showed "t Stat > t Critical one-tail" 8.25 > 1.81 for $\alpha = 0.05$ and 8.25 > 4.14 for $\alpha = 0.001$, p one-tail 0.0000045 < 0.001). This could indicate the consumption of racemic amphetamine (see section S1 for further discussion). Interestingly, amphetamine was found to be enriched with *S*-(+)-enantiomer in Milan (EF_{ww}=0.67±0.16). This suggests either usage of *S*-(+)-amphetamine (prescribed or illicit) or its formation as a result of metabolism of methamphetamine. Indeed, the illicit origin of amphetamine is very likely as methamphetamine was also found to be enriched with the S-(+)-enantiomer (see section 3.1.2).

3.1.2. Methamphetamine

In this study, population-normalised methamphetamine loads were <5 mg day^{-1} 1000 people^{-1} in Bristol and Brussels to a maximum value of 172.4 mg day^{-1} 1000 people^{-1} in Oslo wastewater (Figures 1b and S2, estimated consumptions in Table S12). According to the EMCDDA (EMCDDA 2015), high methamphetamine seizures were reported in Norway. A correlation (not statistically significant) was found between amount seized and loads in wastewater (Baz-Lomba, Salvatore et al. 2016). Zurich wastewater was found to have the second highest methamphetamine loads of 20.2 mg day^{-1} 1000 people^{-1} as a weekly average of eight cities. Estimates in Copenhagen and Brussels were below the overall mean value. Wastewater from other European cities contained low levels. Despite being below the European average

(http://www.emcdda.europa.eu/topics/pods/waste-water-analysis 2016), data from Milan has shown that the methamphetamine load has doubled when compared to data from the same area in 2013-14 and reaching 2012 loads.

Enantiomeric profiling of European wastewater revealed that methamphetamine used in most European locations tested was the enantiopure *S*-(+)-methamphetamine with EF_{ww} ranging from 0.89±0.01 to 1.00±0.00. Norwegian wastewaters were an exception as they contained racemic methamphetamine ($EF_{ww(n=7)}$=0.49±0.02), which also indicated direct disposal of unused (±)-methamphetamine. Indeed, it has been reported by the EMCDDA (EMCDDA 2014) that methamphetamine available in Norway (and in Sweden) is mainly produced from phenylacetone and trafficked as racemate from Lithuania (see section S2 for further information). This is because clandestine production facilities in Lithuania tend to utilise a different synthetic route for methamphetamine production than facilities in Central Europe. Interestingly, since *S*-(+)-methamphetamine is the most potent psychotropic enantiomer (Freeman and Alder 2002) of methamphetamine, one can conclude that despite the lower usage of methamphetamine in Zurich, Copenhagen, Brussels and Milan, the potency of the drug is much higher in these cities than in Oslo.

3.2.MDMA and MDA

The European drug report 2015 stated that 1.8 million Europeans with an age range from 15 and 34 used ecstasy (with MDMA as the main ingredient) in the last year, with a low and stable prevalence trend (EMCDDA 2015). Europe-wide MDMA usage was also estimated using WBE (Thomas, Bijlsma et al. 2012, Ort, van Nuijs et al. 2014). Unfortunately, so far estimations are based on quantification of MDMA as a DTR in wastewater. Such an approach does not allow for accurate evaluation of MDMA consumption against the direct disposal of unused drug. There are two possible solutions: (1) specific metabolic biomarkers should be sought as MDMA is known to metabolise to MDA, DHMA and HMMA (Figure S3) (Castrignanò, Lubben et al. 2016, Gonzalez-Marino, Zuccato et al. 2017), and (2) enantiomeric profiling should be implemented as MDMA undergoes stereoselective metabolism leading to the formation of chiral metabolites (see section S3 for further information).

In the current study, population-normalised MDMA loads ranged from a minimum average value of 3.2 mg day^{-1} 1000 $people^{-1}$ in Castellón to a maximum value of 62.0 mg day^{-1} 1000 $people^{-1}$ in Utrecht (Figures 2 and S2, estimated consumptions also in Table S13). Increasing MDMA loads were found during the weekend in all the countries involved, with the exception of Utrecht that had also high MDMA loads on a weekday. The overall MDMA weekly mean in 2013 was 18 mg day^{-1} 1000 $people^{-1}$ (Ort, van Nuijs et al. 2014). A geographical trend of MDMA loads from North to South was also found. Indeed, Northern European cities (except for Brussels) were mostly above the average. Enantiomeric profiling revealed that MDMA in wastewater is enriched with *R*-(-)-MDMA ($0.32<EF_{ww}<0.40$). This indicates that MDMA retrieved in wastewater comes from consumption, due to the stereoselective metabolism of MDMA in humans. Figure S3 shows expected EF_{ww}s in wastewater for MDMA consumption using the conditions reported in Castrignanò et al (Castrignanò, Lubben et al. 2016). Although illicit MDMA production sites are presumably mainly located in The Netherlands and Belgium (as mentioned in the EMCDDA report (EMCDDA 2015)), MDMA loads in Utrecht and

Brussels were linked to human consumption rather than its direct disposal. In contrast, incidental findings in the wastewater of the city of Utrecht (Emke, Evans et al. 2014) have shown that aberrantly high loads of (±)-MDMA can occur and can be ascribed to disposal of the unconsumed drug.

The hypothesis that MDMA was present in European wastewaters as a result of its consumption was further evidenced by the study of MDA and its chiral signature. MDA can be a drug of abuse itself or a metabolite of MDMA and MDEA (3,4-methylenedioxyethylamphetamine). It is therefore of utmost importance to verify the origin of MDA. It does not have any medical applications and is available on the illicit market as a racemate (Karch and Drummer 2001) ($EF_{illegal\_synth}$=0.5). This is due to its non-stereoselective synthetic route. Similarly to MDMA, MDA's metabolism favours the *S*-(+)-enantiomer (Meyer, Peters et al. 2009). Therefore, if MDA is consumed, it will be excreted in urine enriched with the *R*-(-)-enantiomer (EF_{urine}<0.5). However, if MDA is formed as a result of the metabolism of MDMA or MDEA, it will be present in urine (and in wastewater) enriched with *S*-(+)-enantiomer (Levine 2003, Kasprzyk-Hordern, Kondakal et al. 2010) (EF_{urine}>0.5). In this study, MDEA, for which a new CF was proposed, was not detected in any European location. The highest loads of MDA were recorded in Utrecht with 3.2 mg day^{-1} 1000 $people^{-1}$, followed by Bristol with 1.9 mg day^{-1} 1000 $people^{-1}$ and Oslo with 0.5 mg day^{-1} 1000 $people^{-1}$ at average weekly loads (Table S14). Interestingly, these countries have also high MDMA use, which led us to the conclusion that MDA could be present in wastewater due to consumption of MDMA. In most cases, MDA was found in wastewater enriched with *S*-(+)-enantiomer proving that its presence was associated with the consumption of MDMA, with exception of three days in Bristol, one day in Oslo and in Utrecht when MDA was enriched of the *R*-(-)-form. This could indeed indicate an abuse of MDA. In the case of racemic MDA found in Utrecht for two days, this could indicate a combination of either the consumption of MDA and MDMA (most likely as HMMA data confirmed it) or simply the direct disposal of non-consumed MDA.

As MDA is a minor and not exclusive metabolite of MDMA, other metabolites were also considered as possible DTRs for MDMA consumption: HMA and HMMA. HMA was detected at 3.4 mg day^{-1} 1000 $people^{-1}$ as weekly average in three days of the monitoring week in the Dutch city (Saturday, Sunday and Monday) and at 7.4 mg day^{-1} 1000 $people^{-1}$ in two days in Bristol samples (Sunday and Monday) (Table S15). Because of the low percentage of excretion of HMA after a dose of MDMA, its choice as MDMA DTR could be considered only in the case of high MDMA intake. Indeed, it was only found in those countries reporting the highest levels of MDMA. EF_{ww} showed values close to 0.5 when high HMA loads were detected. However, the relevance of enantioselective analysis is difficult to comment on because of the low number of positive samples for HMA.

HMMA, on the other hand, was found in wastewater at ng/L level in six cities (i.e. no HMMA was detected in Oslo and Milan) (Table S16). HMMA's excretion is 20%, which indicates that it could be used as MDMA's DTR. Due to the stereoselective metabolism of MDMA, HMMA and its glucuronide derivative are formed enriched with *S*-(+)-enantiomer. Interestingly, HMMA sulphate is formed via non-stereoselective route (Schwaninger, Meyer et al. 2012). In this study, HMMA was enriched with the second eluting enantiomer. Assuming the same elution order of MDMA enantiomers for HMA and HMMA under the same chromatographic

conditions, the second-eluting enantiomer could be assigned as *S*-(+)-enantiomer. The expected EF_{ww} would then be >0.5 for HMMA. Therefore, we hypothesize that, if an enrichment of *R*-(-)-MDMA occurred in the case of consumption, the presence of *S*-(+)-HMMA would be observable along with an EF>0.5. Consumption estimates from wastewater analysis were calculated taking into consideration the following DTRs: MDMA itself (CF applied was 1.5 as it was widely used in literature (Zuccato, Chiabrando et al. 2008, Postigo, Lopez de Alda et al. 2010, Nefau, Karolak et al. 2013) even though a new CF of 6.7 was proposed in this study as a result of the most recent excretion data), MDA, HMMA and HMA (see CF in Table S10). The estimates obtained with MDA and HMA showed that these compounds were not suitable as biomarkers of MDMA consumption. Indeed, the estimates calculated by using HMMA were quite superimposable to the parent drug MDMA, except for Oslo.

3.3.Mephedrone

Mephedrone was previously detected in the UK (Castrignanò, Lubben et al. 2016), Italy (González-Mariño, Gracia-Lor et al. 2016), other European cities (Bade, Bijlsma et al. 2017) and in China (Khan, van Nuijs et al. 2014). Its occurrence in wastewater can be only ascribed to illegal disposal or consumption as there is no medical use in Europe (EMCDDA 2011). In this study, a new CF value has been proposed for the first time to allow for the estimation of mephedrone use via WBE. Considering urinary excretion of 15.4%±8.4% as unchanged mephedrone after an oral dose of 150 mg (n=6) (Olesti, Pujadas et al. 2017), CF was set at 6.5. Population-normalised loads ranged throughout a sampling week from 14.9 to 47.7 mg 1000 $people^{-1}$ day^{-1} in the UK (Figures 3 and S2, estimated consumption in Table S17). Increasing loads were found in weekend days rather than weekdays with a mean value of 25.6 ± 12.0 mg 1000 $people^{-1}$ day^{-1}. A similar trend was observed by Castrignanò et al. (Castrignanò, Lubben et al. 2016), classifying mephedrone as a recreational drug like MDMA. Furthermore, mephedrone was found to be enriched with the *R*-(+)-enantiomer in wastewater ($EF_{ww\ in\ 2014\ (n=6)}$=0.57±0.02 and $EF_{ww\ in\ 2015\ (n=4)}$=0.57±0.04). This indicates that mephedrone was consumed rather than directly disposed (Castrignanò, Mardal et al. 2017) (see section S4 for further information).

3.4.Other drugs and precursors

The analysis of drug precursors, such as norephedrine, ephedrine and pseudoephedrine (referred in the text as ephedrines), was performed only for Oslo, Bristol, Utrecht (only norephedrine) and Milan (see section S5 for further information).

Mean population-normalised norephedrine loads were 51 mg 1000 $people^{-1}$ day^{-1} in Oslo (probably linked to methamphetamine's metabolism), 7.1 mg 1000 $people^{-1}$ day^{-1} in Milan and 3.4 mg 1000 $people^{-1}$ day^{-1} in Bristol (Table S18-Figure 4c). Norephedrine was not detected in wastewater from Utrecht. EFs were 0.48±0.04, 0.56±0.11 and 1.00±0.00 (due to <MQL values for the first eluting enantiomer), respectively.

Only two stereoisomers of ephedrine were found in European wastewaters: *1R,2S*-(-)-ephedrine and *1S,2S*-(+)-pseudoephedrine. Population-normalised *1R,2S*-(-)-ephedrine loads were 0.7 mg 1000 $people^{-1}$ day^{-1} in Oslo, 3.4 mg 1000 $people^{-1}$ day^{-1} in Milan and 0.6 mg 1000 $people^{-1}$ day^{-1} in Bristol (Table S19-Figure 4a). Mean population-normalised *1S,2S*-(+)-pseudoephedrine

loads were 21.2 mg 1000 people^{-1} day^{-1} in Oslo, 35.7 mg 1000 people^{-1} day^{-1} in Milan and 96.4 mg 1000 people^{-1} day^{-1} in Bristol (Table S19-Figure 4b).

Chiral PMA (para-methoxyamphetamine), a phenylisopropylamine with hallucinogenic properties, has no legitimate therapeutical use. It is abused alone or in combination with MDMA or PMMA. Seizures have been reported in several European countries, including Belgium, Denmark, Spain, the Netherlands and the UK. However, it was not found in wastewater from any studied city. This is also in accordance with Kinyua et al. (Kinyua, Covaci et al. 2015).

3.5. Consumption estimates of (meth)amphetamine and ephedrines corrected for legal use: a case study in England

In England, legal amphetamine prescriptions in 2015 were as follows: 17.8 kg/year of *S*-(+)-amphetamine (73.4% correction from 23.7 kg/year as dexamfetamine sulphate (Team, Centre et al. 2016) to the free base) and 20.3 kg/year as *S*-(+)-amphetamine (29.7% correction from 68.4 kg/year as lisdexamfetamine dimesylate (Team, Centre et al. 2016) to the free base) (Table 1). Taking into account urinary excretion, the annual amount excreted as *S*-(+)-amphetamine is calculated as 5.2 kg from dexamfetamine sulphate consumption and 8.4 kg from lisdexamfetamine dimesylate. Moreover, 1.3 kg of *R*-(-)-amphetamine was excreted in 2015 from 9.7 kg/year of prescribed selegiline (Team, Centre et al. 2016). As a result, the contribution of legal prescribed and excreted amphetamine to wastewater in the WWTP considered in the study was 1.6 and 0.10 mg day^{-1} 1000 people^{-1} of *S*-(+)- and *R*-(-)-amphetamine, respectively (this does not consider legally purchased drugs traded illegally). Consumption estimates from wastewater analysis were back-calculated by using amphetamine and norephedrine as DTRs (3.3 and 44.7 as corresponding CFs). Despite the good agreement between estimates obtained with considered DTRs, norephedrine is not recommended as a biomarker for amphetamine use as it can result from other sources (e.g. disposal of norephedrine and metabolism of ephedrine and methamphetamine). In relation to these findings, the presence of amphetamine in Bristol was linked to an illegal use of the substance since the contribution of estimates from the legal sources was negligible (Table 1).

Regarding methamphetamine, 2.7 kg/year of the *R*-(-)-enantiomer was excreted into wastewater as a result of 9.7 kg/year of selegiline intake (Team, Centre et al. 2016). Thus, by normalising the data with the population equivalent served by the local WWTP in England, 0.18 mg day^{-1} 1000 people^{-1} of *R*-(-)-methamphetamine (originating from selegiline consumption) was estimated in the studied location. Consumption estimates were performed considering methamphetamine itself, amphetamine and norephedrine as DTRs (see CFs in Table S10). The estimates obtained with amphetamine and norephedrine as DTR were 100-fold higher than the estimate calculated from methamphetamine. 2.70 mg day^{-1} 1000 people^{-1} of (±)-methamphetamine, of which 1.8 as *R*-(-)-enantiomer, were estimated by using methamphetamine as DTR, suggesting that its presence was associated mainly with illegal use.

The estimates of the legal use of ephedrines in England in 2015 are as follows (Table 1):

- ephedrine: 0.83 kg/year as hydrochloride (or 0.62 kg/year as free base) resulting in annual excretion of 0.46 kg of ephedrine in England;

- pseudoephedrine: 253.54 kg/year as hydrochloride (or 223.12 kg/year as *1S,2S*-(+)-enantiomer) resulting in annual excretion of 196.34 kg of *1S,2S*-(+)-pseudoephedrine in England;
- norephedrine: 0.35/year and 0.02 kg/year excreted as a result of dexamfetamine sulphate and ephedrine consumption, respectively.

Furthermore, the metabolism of selegiline produces 0.62% (n=4) of (*1S,2R*)-(+)-ephedrine, 0.04% (n=4) as (*1R,2R*)-(-)-pseudoephedrine and 0.12% (n=4) as (*1S,2R*)-(+)-norephedrine (Shin 1997). In 2015 in England, 0.06 kg/year of (*1S,2R*)-(+)-ephedrine, 0.004 kg/year of (*1R,2R*)-(-)-pseudoephedrine and 0.011 kg/year as (*1S,2R*)-(+)-norephedrine were excreted as a result of 9.72 kg/year of selegiline intake (Team, Centre et al. 2016).

Final estimates, normalised with local WWTP, were 0.034, 10.61 and 0.02 mg day^{-1} 1000 $people^{-1}$ of ephedrine, pseudoephedrine and norephedrine respectively (CFs in Table S10). For Bristol, consumption estimates were in agreement with the legal usage of ephedrine when ephedrine itself was used as DTR and discordant in the case of pseudoephedrine and norephedrine (most likely due to their availability on the OTC market).

4. Conclusions

This study was the first to spatially and temporally assess the enantiomeric profiling of chiral illicit drugs in wastewater serving 4.9 million people in eight European cities. Spatial variations in drug loads were observed across Europe with higher use of amphetamine in Northern European cities, revealing a general enrichment of *R*-(-)-amphetamine in wastewater. The chiral signature of amphetamine revealed that it is present in wastewater as a result of its consumption. High methamphetamine loads were detected in Oslo, where racemic methamphetamine was present, likely due to different trafficking routes from the Baltic countries, rather than Western and Central Europe. The more potent *S*-(+)-methamphetamine was the predominant enantiomer found in wastewater from the other European cities tested, which indicates distribution of enantiopure *S*-(+)-methamphetamine on the illicit market. It could suggest that direct comparison of methamphetamine loads in Oslo and the other European cities should not be undertaken without considering its chiral signature and the different potency of individual enantiomers. The analysis of precursors was compatibly ascribed to their medical use. MDMA was commonly enriched with *R*-(-)-enantiomer in studied European cities, which indicates consumption rather than disposal of the unused drug. MDA was commonly found to be enriched with *S*-(+)-enantiomer, which indicates that its presence in European wastewaters originates from MDMA metabolism (especially during weekends) rather than consumption of MDA itself. However, on a few occasions (UK and The Netherlands), MDA was found to be enriched with *R*-(-)-enantiomer, which indicates its consumption. As MDA is a minor metabolite of MDMA, other metabolites were considered as possible MDMA DTRs, namely HMA and HMMA. HMMA was found to be a suitable MDMA DTR. Furthermore, its chiral signature indicated its enrichment with *S*-(+)-enantiomer, which confirms its origin from MDMA metabolism. Population-normalised mephedrone loads were up to 47.7 mg 1000 $people^{-1}$ day^{-1} in wastewater in the UK, where an enrichment of *R*-(+)-enantiomer suggested stereoselective metabolism in humans, indicating consumption rather than direct disposal.

Contributions

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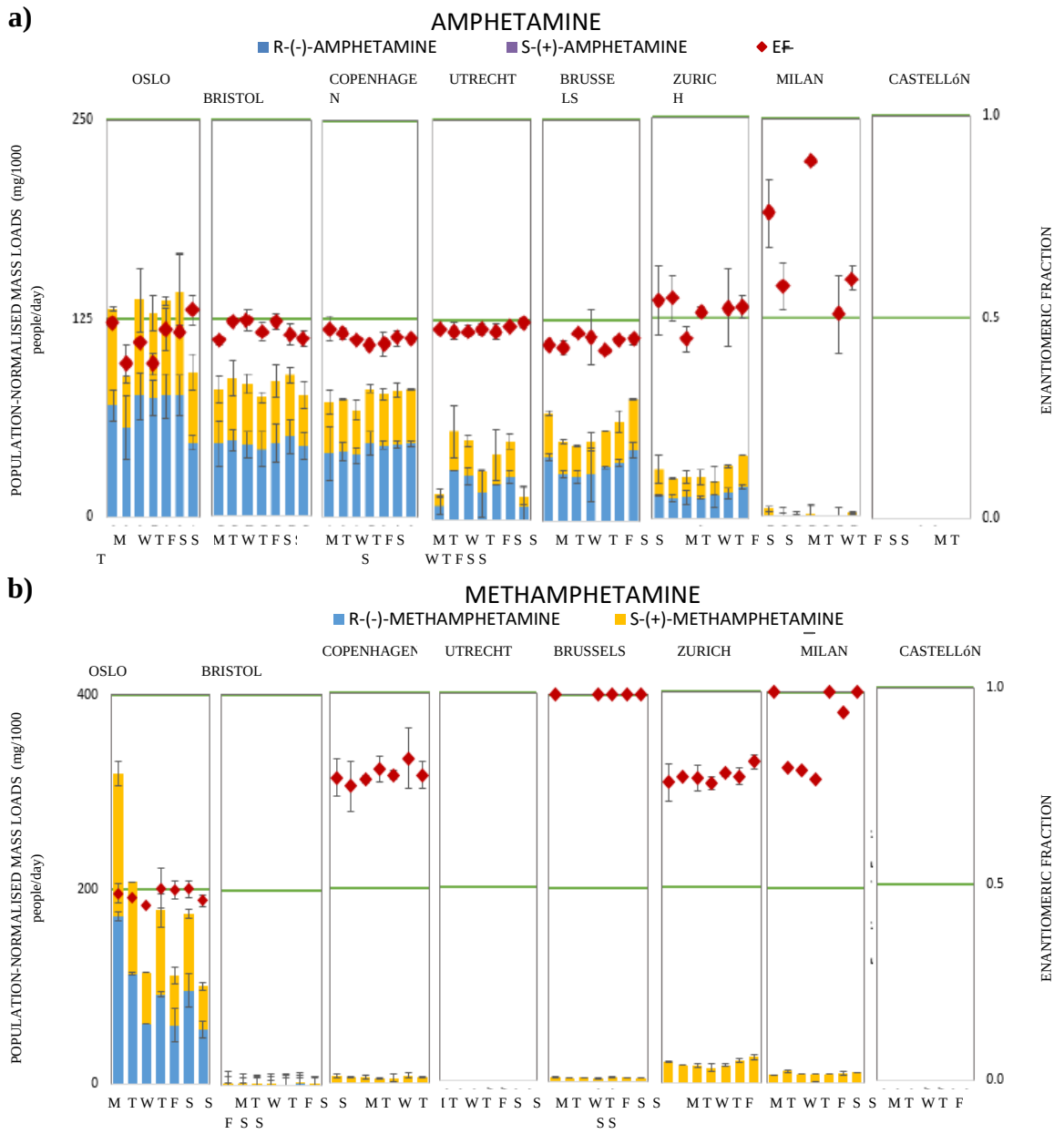

Figure 1 Amphetamine (a) and methamphetamine (b): population-normalised mass loads and enantiomeric fraction values in a week monitoring campaign (M, T, W, T, F, S, S indicate week days). The absence of the bars indicates 'not detected'.

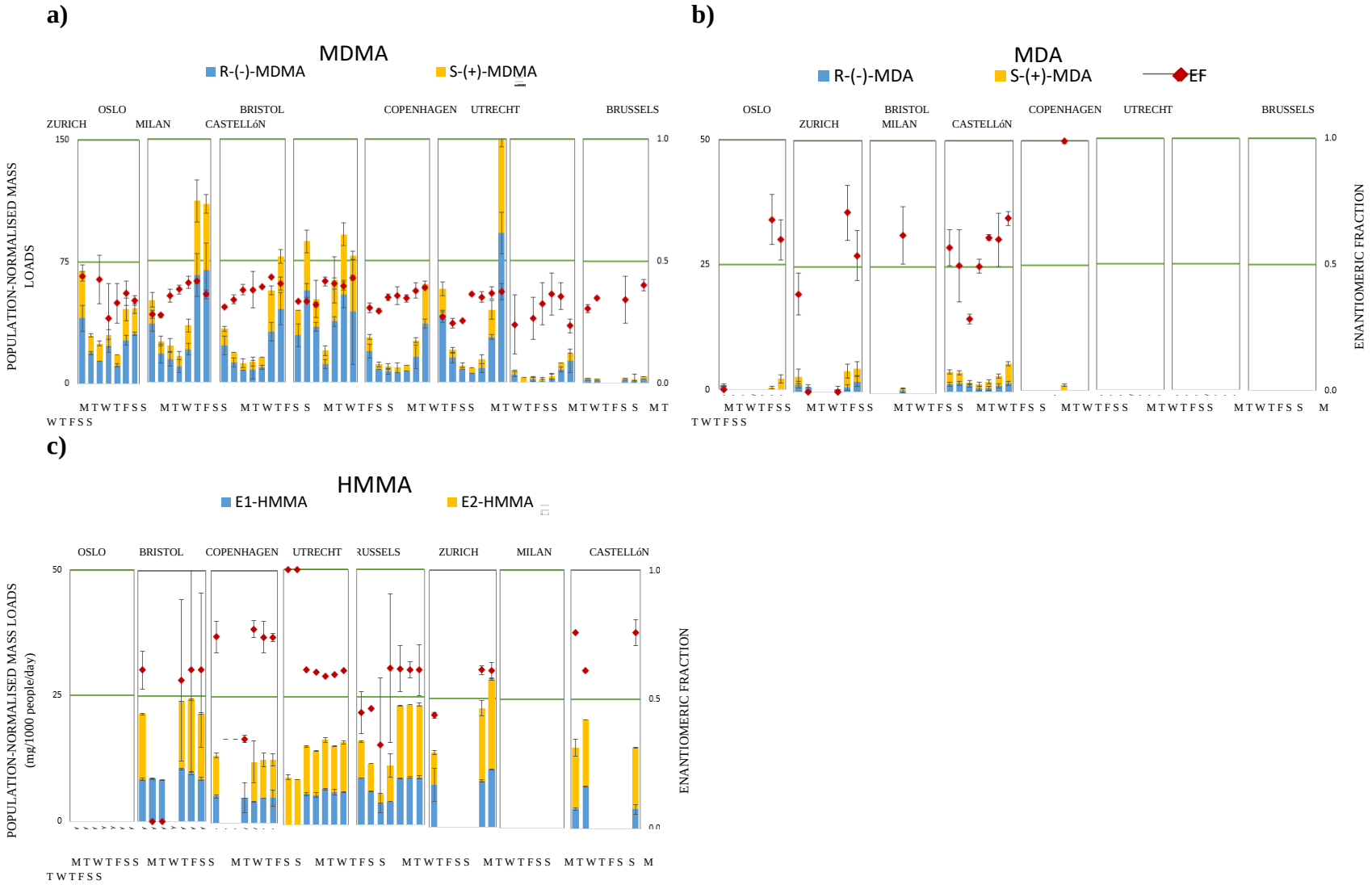

Figure 2 MDMA (a), MDA (b) and HMMA (c): population-normalised mass loads and enantiomeric fraction values in a week monitoring campaign (M, T, W, T, F, S, S indicate week days). For HMMA: EF values are reported assuming that the first-eluting enantiomer is *R*-(-)-HMMA and the second one is *S*-(+)-HMMA. The absence of the bars indicates 'not detected'.

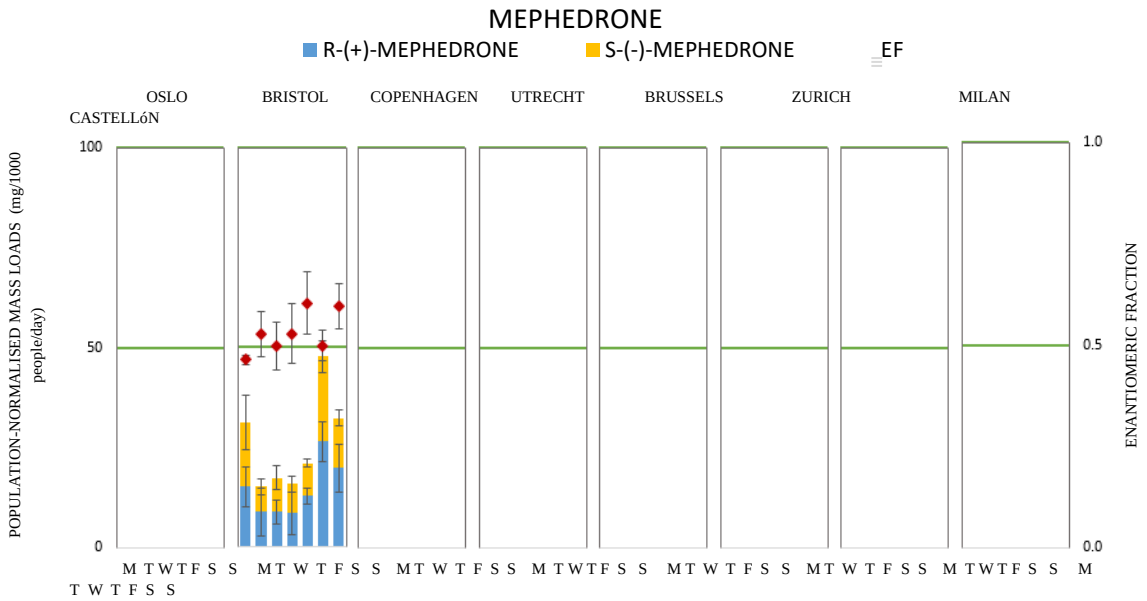

Figure 3 Population-normalised mephedrone loads and enantiomeric fraction values in a week monitoring campaign (M, T, W, T, F, S, S indicate week days). The absence of the bars indicates ‘not detected’.

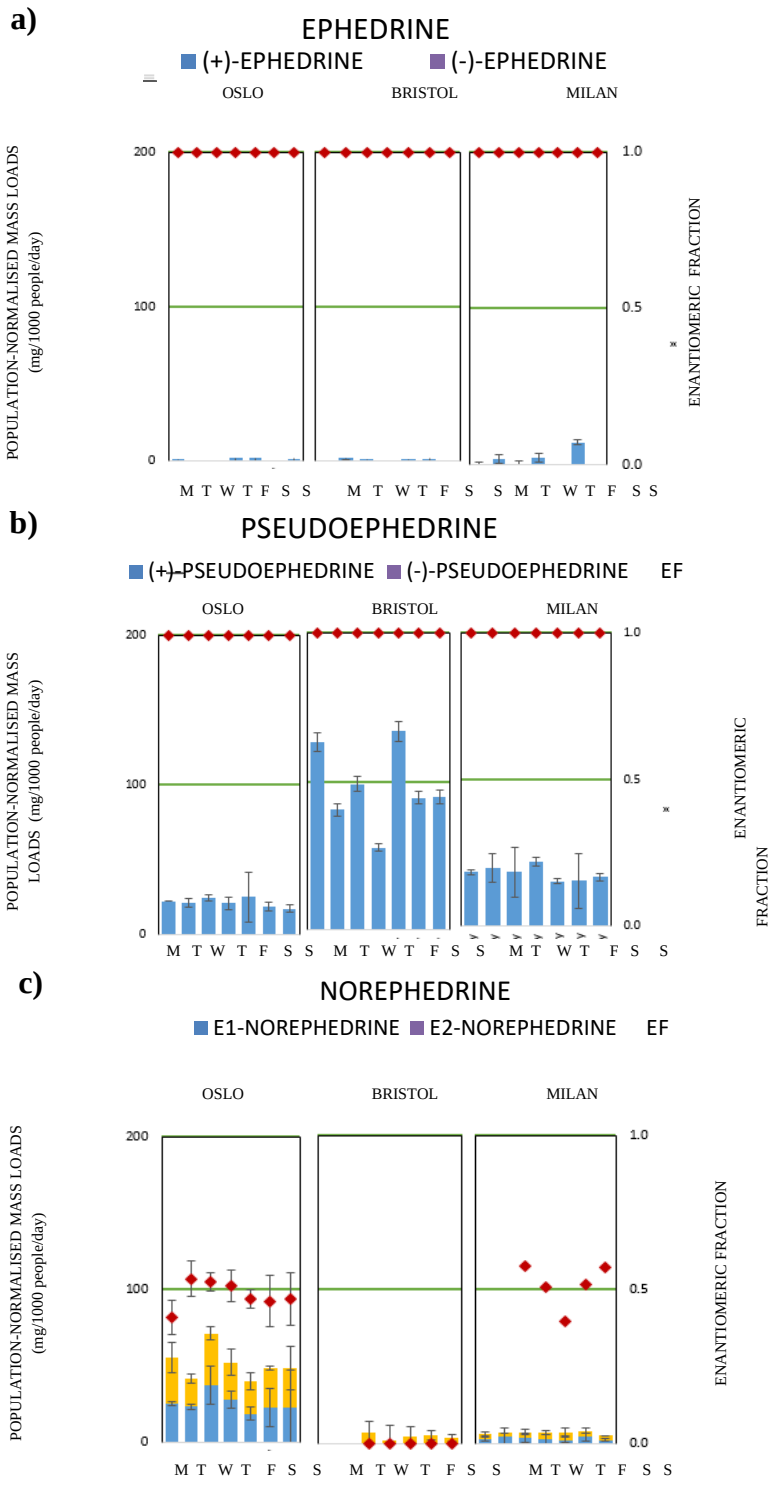

Figure 4 Ephedrine (a), pseudoephedrine (b) and norephedrine (c): population-normalised mass loads and enantiomeric fraction values in a week monitoring campaign (M, T, W, T, F, S, S indicate week days). The absence of the bars indicates 'not detected'.

Table 1 Consumption estimates and contribution from legal use in England in 2015. The following information is provided: biomarkers of drugs of abuse and precursors (AMP=amphetamine, METH=methamphetamine, EPH=ephedrine, PSEUDOEPH=pseudoephedrine and NE=norephedrine), parent compound or metabolite used as drug target residue (DTR), information derived from the legal use of drugs in England in 2015 and consumption estimates calculated from official health statistics in relation to the population served by the wastewater treatment plant in the study (Bristol) and from wastewater analysis ([a] calculated from weekly average loads of considered DTR).

Drug	DTR	Legal Use in England in 2015				Consumption estimates (mg day^{-1} 1000 people^{-1})	
		From metabolism of prescribed pharmaceuticals	**Amount prescribed in England in 2015 (kg/year)**	**Excretion (%)**	**Amount excreted as metabolite in England in 2015 (kg/year)**	**Health national statistics (2015)**	**Wastewater analysis (2015)[a]**
AMP	AMP	73.4% *S*-(+)-AMP base from dexamphetamine sulfate	23.67 kg/year as dexamfetamine sulfate (or 17.38 kg/year as *S*-(+)-AMP)	30.0% in neutral pH condition, up to 74.0% in acidic and 1.0% in alkaline urine (Baselt 2008)	5.21 kg in neutral pH urine (or 12.86 kg in case of acidic urine or 0.17 kg in case of alkaline urine)	0.32 as *S*-(+)-AMP	272.7 as (±)-AMP, of which 120.1 as *S*-(+)-AMP; 213.7 as NE
	NE	Lisdexamfetamine	68.35 kg/year as lisdexamfetamine dimesylate (or 20.30 kg/year as *S*-(+)-AMP)	41.5% *S*-(+)-AMP (Krishnan, Pennick et al. 2008)	8.42 kg	1.26 as *S*-(+)-AMP	
		Selegiline	9.72 kg/year	3.06±1.10 (n=4) as *R*-(-)-AMP (Shin 1997), 13.5% (Cody 2002)	1.31 kg as *R*-(-)-AMP (using 13.5% as excretion)	0.10 as *R*-(-)-AMP	
METH	METH AMP NE	Selegiline	9.72 kg/year	36.96±8.17 (n=4) as *R*-(-)-METH (Shin 1997); 27.5% (Cody 2002)	2.67 kg as *R*-(-)-METH	0.18 as *R*-(-)-METH	2.7 as (±)-METH, of which 1.8 as *R*-(-)-METH; 1661.1 as (±)-AMP and 94.2 as NE
EPH	EPH	EPH	0.83 kg/year as EPH hydrochloride (or 0.62 kg/year as EPH)	75% used as average of excretion	0.46 kg	0.03 as EPH	0.8 as 1*R*,2*S*-(−)-EPH, 93.2 as (±)-NE
	NE	Selegiline	9.72 kg/year	0.62±0.29 (n=4) as (1*S*,2*R*)-(+)-EPH (Shin 1997)	0.06 kg as (1*S*,2*R*)-(+)-EPH	0.004 as (1*S*,2*R*)-(+)-EPH	
PSEUDO EPH	PSEUDO EPH	PSEUDOEPH	253.54 kg/year as PSEUDOEPH hydrochloride (or 223.12 kg/year as 1*S*,2S-(+)-PSEUDOEPH)	88.0% (Baselt 2008)	196.34 kg as 1*S*,2S-(+)-PSEUDOEPH	10.61 as 1*S*,2S-(+)-PSEUDOEPH	106.0 as 1*S*,2S-(+)-PSEUDOEPH
		Selegiline	9.72 kg/year	0.04±0.03 (n=4) as (1*R*,2*R*)-(-)-PSEUDOEPH (Shin 1997)	0.004 kg as (1*R*,2*R*)-(-)-PSEUDOEPH	0.0002 as (1*R*,2*R*)-(-)-PSEUDOEPH	
NE	NE	Dexamfetamine	23.67 kg/year as dexamfetamine sulfate	2.0% in neutral pH condition (Baselt 2008)	0.35 kg in neutral pH urine	0.02 as NE	4.1 as (±)-NE
		EPH	0.83 kg/year as EPH hydrochloride	4.0% (Baselt 2008)	0.02 kg	0.0015 as NE	
		Selegiline	9.72 kg/year	0.12±0.05 (n=4) as (1*S*,2*R*)-NE (Shin 1997)	0.011 kg as (1*S*,2*R*)-NE	0.0008 as (1*S*,2*R*)-NE	

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Supplementary Material

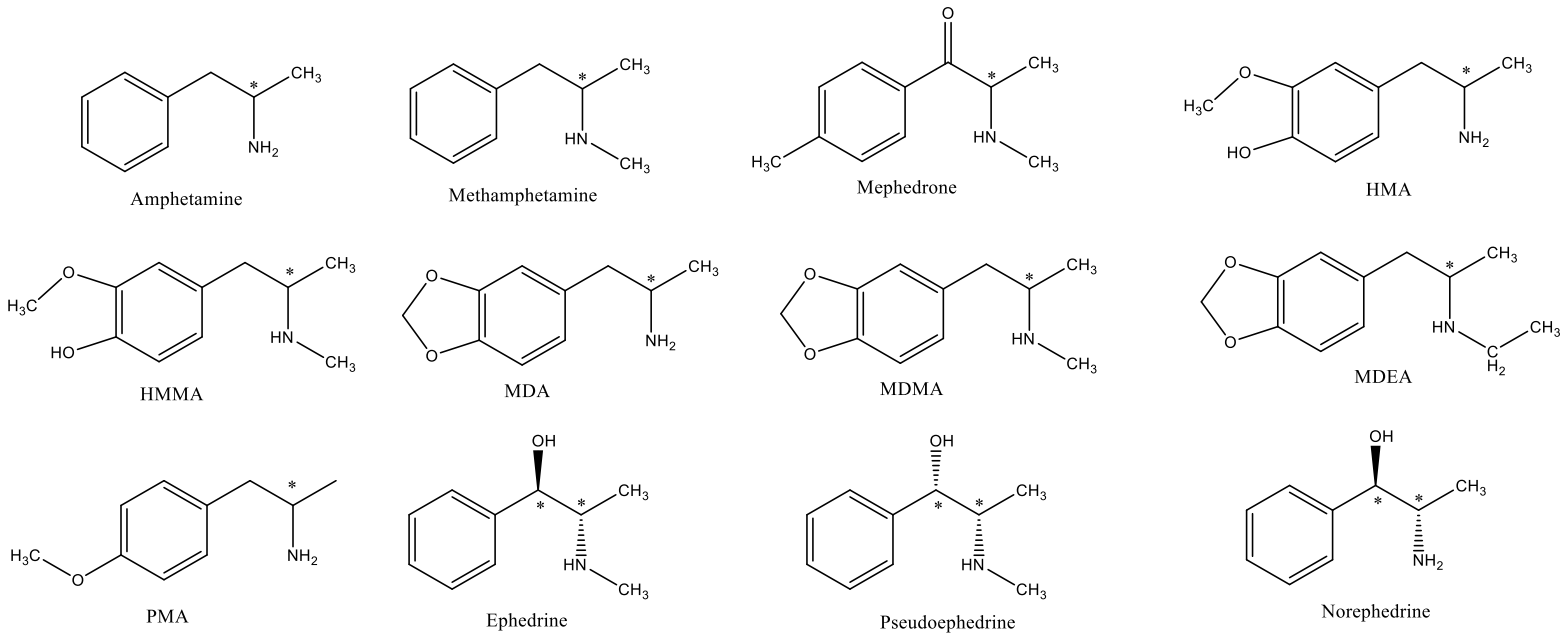

Figure S1 Chiral analytes selected in the study.

Table S1 Selected analytes and their properties (MW molecular weight, Exp experimental, Pred predicted, [a] extracted from [38], [b] predicted using ACD/labs software (http://www.chemspider.com).

Compound	CAS	Formula	MW	pK_a		LogP		LogD[b]		Supplier
				Exp.[a]	Pred.[a]	Exp.[a]	Pred.[b]	pH 5.5	pH 7.4	
(±)-Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1 (20°)	10.01	1.85	1.81±0.20	-1.28	-0.63	LGC(Cerilliant product)
(±)-Methamphetamine	4846-07-5	$C_{10}H_{15}N$	149.2	9.87 (25°)	10.21	2.07	1.94±0.21	-1.15	-0.79	LGC (Cerilliant product)
(±)-Mephedrone	1189726-22-4	$C_{11}H_{15}NO$	177.7	-	-	-	1.86±0.31	-0.03	1.55	Sigma-Aldrich (Cerilliant product)
PMA (p-Methoxyamphetamine)	3706-26-1	$C_{10}H_{15}NO$	165.0	-	-	-	1.72±0.23	-1.36	-0.76	LGC
(±)-MDA (3,4-methylenedioxyamphetamine)	4764-17-4	$C_{10}H_{13}NO_2$	179.2	9.67 (25°)	10.01	1.64	1.67±0.27	-1.41	-0.77	LGC (Cerilliant product)
(±)-MDMA (3,4-methylenedioxymethamphetamine)	42542-10-9	$C_{11}H_{15}NO_2$	193.2	-	10.143	- 1.65,1.86	1.81±0.27	-1.29	-0.90	LGC
(±)-MDEA (3,4-methylenedioxyethylamphetamine)	82801-81-8	$C_{12}H_{17}NO_2$	207.3	-	-	-	2.66±0.27	-0.42	0.30	LGC (Cerilliant product)
D,L-HMA (d,l-4-Hydroxy-3-methoxyamphetamine)	13062-61-8	$C_{10}H_{15}NO_2$	181.2	-	-	-	-	-	-	Kinesis
D,L-HMMA (d,l-4-Hydroxy-3-methoxy-methamphetamine)	438625-58-2	$C_{11}H_{17}NO_2$	195.2	-	-	-	1.41	-1.68	-1.24	Kinesis
(±)-Ephedrine	50-98-6	$C_{10}H_{15}NO$	165.2	10.3 (0°)	13.89, 9.52	1.13	1.05±0.27	-1.99	-0.96	Sigma-Aldrich
(±)-Norephedrine	154-41-6	$C_9H_{13}NO$	151.2	9.44 (20°)	13.9, 9.37	0.67	0.81±0.26	-2.21	-1.07	Sigma-Aldrich

Table S2 Selected cities in the study, population and flow data.

City	Bristol	Oslo	Milan	Utrecht	Castellon	Brussels	Zurich	Copenhagen
Country	UK	Norway	Italy	The Netherlands	Spain	Belgium	Switzerland	Denmark
Residential population	886650	580639	1100000	300000	180690	954000	410000	531000
Day	**Flow in m3/day**							
Monday	197493.3	254570.5	597470.0	45970.0	37469.0	234774.0	177167.0	148724.0
Tuesday	204490.8	252721.5	423110.0	44580.0	40476.0	359951.0	160912.0	150936.0
Wednesday	198950.4	333480.1	403240.0	47740.0	50228.0	234264.0	157084.0	147175.0
Thursday	197523.0	308279.1	412310.0	45030.0	49161.0	235442.0	161005.0	144840.0
Friday	252682.2	277449.7	402240.0	49530.0	43728.0	234906.0	161427.0	145197.0
Saturday	220687.2	256766.4	403020.0	46030.0	38301.0	233096.0	200010.0	137793.0
Sunday	193194.0	250383.9	422690.0	46900.0	37243.0	230375.0	243013.0	137244.0

Table S3 MRM transitions selected for studied analytes.

Compound	CV/CE[a]	MRM1 (quantification)	CV/CE[a]	MRM2 (confirmation)	CV/CE[a]	MRM3 (confirmation)	MRM1/MRM2 ratio ± SD	MRM1/MRM3 ratio ± SD	IS
Amphetamine	18/16	136.16 > 91.1	18/8	136.16 > 119.1	-	-	1.2 ± 0.1	-	Amphetamine-D5
Methamphetamine	24/19	150.2 > 91.1	24/10	150.2 > 119.1	-	-	1.8 ± 0.1	-	Methamphetamine-D5
MDA	21/11	180.0 > 163.1	21/22	180.0 > 105.1	-	-	2.6 ± 0.4	-	MDA-D5
MDMA	24/13	194.1 > 163.1	24/24	194.1 > 105.1	-	-	2.1 ± 0.1	-	MDMA-D5
MDEA	28/13	208.1 > 163.1	28/27	208.1 > 105.1	-	-	2.1 ± 0.2	-	MDEA-D5
HMA	6/14	182.1 > 165.0	6/24	182.1 > 105.0	6/18	182.1 > 133.0	1.8 ± 0.7	2.4 ±1.4	Amphetamine-D5
HMMA	16/12	196.1 > 165.0	16/26	196.1 > 105.0	16/22	196.1 > 133.0	3.1 ± 0.6	3.8 ±0.6	Methamphetamine-D5
Mephedrone	10/12	178.1 > 160.1	10/22	178.1 > 145.0	10/22	178.1 > 119.0	1.6 ± 0.2	8.5 ±2.1	Mephedrone-D3
p-Methoxyamphetamine (PMA)	20/20	166.0 > 121.0	20/20	166.0 > 149.0	-	-	12.5 ± 1.5	-	MDA-D5
Ephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	-	-	7.4 ± 0.8	-	1S, 2R-(+)-ephedrine-D3
Pseudoephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	-	-	6.9 ± 0.6	-	1S, 2R-(+)-ephedrine-D3
Norephedrine	23/10	152.1 > 134.1	23/16	152.1 > 117.1	-	-	3.1 ± 0.4	-	1S, 2R-(+)-ephedrine-D3

ISs	CV/CE[a]	MRM1 (quantification)
Amphetamine-D_5	22/16	141.0 > 92.9
Methamphetamine-D_5	28/12	155.1 > 121.0
Mephedrone-D_3	30/22	181.1 > 163.1
MDA-D_5	21/11	185.1 > 168.1
MDMA-D_5	26/13	199.1 > 165.1
MDEA-D_5	28/13	213.1 > 163.0
1S,*2R*-(+)-Ephedrine-D_3	23/18	169.2 > 151.0

[a]CV, cone voltage (V); CE, collision energy (eV)

Table S4 Validation parameters - retention time, relative retention time, linearity range, correlation coefficient obtained from calibration curve and instrumental and method limits of detection and instrumental and method limits of quantification.

Compound	R_t (min)	Rel. R_t	Sample diluent				WWTP influent	
			Linearity range in mobile phase (µg/L)	R^2	$IDL_{S/N}$ (µg/L)	$IQL_{S/N}$ (µg/L)	MDL (ng/L)	MQL (ng/L)
R-(-)-Amphetamine	15.5 ±0.3	0.1	0.125-500	0.9987	0.12	0.50	0.76	2.89
S-(+)-Amphetamine	22.6 ±0.4	0.2	0.125-500	0.9988	0.12	0.50	0.72	2.87
R-(-)-Methamphetamine	14.5 ±0.4	0.3	0.050-500	0.9989	0.05	0.12	0.26	0.66
S-(+)-Methamphetamine	16.5 ±0.4	0.3	0.050-500	0.9994	0.05	0.12	0.30	0.74
E1-Mephedrone	16.5 ±0.4	0.3	0.250-500	0.9990	0.25	0.50	1.30	2.60
E2-Mephedrone	21.0 ±0.5	0.2	0.250-500	0.9993	0.25	0.50	0.66	2.63
R-(-)-MDA	28.1 ±0.5	0.2	0.500-500	0.9991	0.50	2.50	2.80	14.02
S-(+)-MDA	47.4 ±0.8	0.4	0.500-500	0.9980	0.50	2.50	2.50	12.48
R-(-)-MDMA	21.9 ±0.5	0.2	0.050-500	0.9992	0.05	0.25	0.29	1.44
S-(+)-MDMA	32.9 ±0.5	0.1	0.050-500	0.9994	0.05	0.25	0.27	1.35
E1-MDEA	19.0 ±0.5	1.8	0.125-500	0.9994	0.12	0.25	0.65	1.30
E2-MDEA	21.0 ±0.5	0.2	0.125-500	0.9995	0.12	0.25	0.66	1.32
E1-HMA	17.7 ±0.4	0.4	2.500-500	0.9900	2.50	5.00	11.83	23.65
E2-HMA	34.3 ±0.5	0.8	2.500-500	0.9903	2.50	5.00	11.28	22.55
E1-HMMA	15.9 ±0.4	2.5	0.250-500	0.9982	0.25	0.50	1.39	2.79
E2-HMMA	18.6 ±0.5	2.5	0.250-500	0.9974	0.25	0.50	1.13	2.27
E1-Norephedrine	13.6 ±0.3	0.4	0.125-500	0.9981	0.12	0.25	0.56	1.11
E2-Norephedrine	15.1 ±0.4	2.2	0.125-500	0.9983	0.12	0.25	0.64	1.28
E1-PMA	21.3 ±0.5	0.5	0.125-500	0.9964	0.12	0.25	0.66	1.32
E2-PMA	36. 8 ±0.4	1.4	0.125-500	0.9994	0.12	0.25	0.58	1.17
(+)-Ephedrine	12.3 ±0.3	0.6	1.000-500	0.9974	1.00	5.00	5.91	29.53
(-)-Ephedrine and (-)-Ψephedrine	13.4 ±0.	0.5	0.500-1000	0.9975	0.50	1.00	2.40	4.81
(+)-Ψephedrine	32.94 ±0.8	1.9	1.000-500	0.9903	1.00	5.00	5.60	27.99

Table S5 Validation parameters - method precision.

Analyte	**Intra-day RSD% (n=4)**									**Inter-day RSD% (n=3)**		
	25 ng/L** D 1*	25 ng/L D 2	25 ng/L D 3	250 ng/L D 1	250 ng/L D 2	250 ng/L D 3	2500 ng/L D 1	2500 ng/L D 2	2500 ng/L D 3	25 ng/L	250 ng/L	2500 ng/L
R-(-)-Amphetamine	3.3	2.5	4.6	5.2	14.7	10.8	6.2	3.9	6.2	3.5	10.2	5.4
S-(+)-Amphetamine	3.1	4.3	12.6	1.4	6.5	4.7	3.8	7.0	7.3	6.7	4.2	6.0
R-(-)-Methamphetamine	8.9	6.7	9.3	3.4	7.0	8.3	4.8	5.2	5.4	8.3	6.2	5.1
S-(+)-Methamphetamine	6.8	3.6	15.4	1.2	5.5	4.0	2.7	2.9	4.2	8.6	3.6	3.3
E1-Mephedrone	9.8	13.7	14.1	3.6	6.8	14.6	3.7	10.0	5.6	12.5	8.3	6.4
E2-Mephedrone	10.7	12.0	4.6	5.2	12.9	8.4	9.2	3.7	2.8	9.1	8.8	5.2
R-(-)-MDA	1.7	6.6	9.7	3.0	3.4	5.7	0.1	7.7	1.1	6.0	4.0	3.0
S-(+)-MDA	4.4	3.8	7.8	2.6	6.7	5.3	7.2	3.7	4.5	5.3	4.9	5.1
R-(-)-MDMA	7.0	1.8	4.0	5.8	4.6	3.9	3.4	1.5	6.5	4.3	4.8	3.8
S-(+)-MDMA	1.0	1.9	6.9	0.6	3.1	2.9	1.2	2.8	0.7	3.3	2.2	1.6
E1-MDEA	6.9	6.2	3.0	5.1	8.5	7.8	4.7	2.2	4.3	5.4	7.1	3.7
E2-MDEA	6.0	6.3	2.8	1.4	9.2	4.9	8.3	1.4	1.7	5.0	5.2	3.8
E1-HMA	4.4	5.1	1.6	7.6	1.1	4.4	6.4	6.0	5.9	3.7	4.4	6.1
E2-HMA	5.2	4.8	12.6	3.8	2.0	5.0	7.0	6.5	6.0	7.5	3.6	6.5
E1-HMMA	7.4	7.6	7.5	2.8	3.8	6.0	4.1	2.7	0.3	7.5	4.2	2.4
E2-HMMA	4.7	6.4	3.6	2.1	2.1	6.2	2.9	3.1	3.6	4.9	3.5	3.2
E1-Norephedrine	7.3	3.8	1.3	2.8	3.0	7.3	4.4	3.0	7.4	4.1	4.3	5.0
E2-Norephedrine	5.7	4.6	6.3	3.1	3.9	6.1	2.2	2.1	3.5	5.5	4.3	2.6
E1-PMA	7.7	4.8	8.3	1.4	4.4	3.7	3.8	4.3	5.3	6.9	3.2	4.5
E2-PMA	6.2	8.8	11.6	7.8	4.6	6.6	1.7	3.9	2.9	8.9	6.3	2.8
(+)-Ephedrine	5.3	16.5	9.8	5.0	4.5	6.6	7.2	2.8	3.3	10.5	5.4	4.4
(-)-Ephedrine and (-)-Ψephedrine	8.3	14.8	5.2	1.8	0.8	5.4	5.7	1.0	3.3	9.4	2.7	3.3
(+)-Ψephedrine	2.8	2.5	6.2	5.8	1.3	9.4	2.9	2.0	1.7	3.8	5.5	2.2

*-D indicates day

Table S6 Validation parameters -instrumental precision.

Analyte	**Intra-day RSD% (n=4)**									**Inter-day RSD% (n=3)**		
	5 µg/L** D 1*	5 µg/L D 2	5 µg/L D 3	50 µg/L D 1	50 µg/L D 2	50 µg/L D 3	500 µg/L D 1	500 µg/L D 2	500 µg/L D 3	5 µg/L	50 µg/L	500 µg/L
R-(-)-Amphetamine	4.8	5.8	3.0	2.3	3.1	0.1	3.9	4.7	3.1	4.5	1.9	3.9
S-(+)-Amphetamine	3.7	5.3	6.5	4.6	3.3	4.3	3.2	4.1	3.4	5.2	4.1	3.6
R-(-)-Methamphetamine	6.0	5.8	6.3	3.9	5.5	2.3	3.0	5.1	2.8	6.0	3.9	3.7
S-(+)-Methamphetamine	2.4	2.3	7.7	2.7	0.7	2.1	1.1	4.8	3.4	4.1	1.8	3.1
E1-Mephedrone	9.3	6.7	5.5	1.9	5.7	5.4	2.9	5.5	4.4	7.1	4.3	4.3
E2-Mephedrone	3.5	6.7	1.1	3.6	2.5	2.7	9.3	4.3	2.2	3.8	3.0	5.2
R-(-)-MDA	6.9	1.3	2.7	0.4	5.6	0.1	1.5	0.3	1.6	3.6	2.1	1.1
S-(+)-MDA	5.7	3.2	6.4	8.0	8.9	3.1	0.3	1.1	6.1	5.1	6.7	2.5
R-(-)-MDMA	2.5	5.5	2.0	1.8	6.4	3.9	4.8	3.7	6.1	3.3	4.0	4.9
S-(+)-MDMA	3.5	1.1	4.3	0.5	1.8	1.3	2.5	1.5	2.7	3.0	1.2	2.3
E1-MDEA	8.6	5.3	5.9	2.2	3.8	1.1	6.1	4.3	0.1	6.6	2.4	3.5
E2-MDEA	3.6	2.3	10.3	5.3	1.1	0.4	5.6	1.9	0.7	5.4	2.3	2.7
E1-HMA	11.3	5.6	6.3	5.3	6.7	9.1	7.4	4.9	2.1	7.7	7.1	4.8
E2-HMA	6.1	1.7	1.1	3.1	0.4	2.5	8.9	6.3	9.0	3.0	2.0	8.1
E1-HMMA	5.3	8.3	4.1	0.8	6.5	6.6	8.2	4.2	1.7	5.9	4.6	4.7
E2-HMMA	6.6	5.7	9.4	2.4	3.3	7.4	3.8	4.0	4.6	7.2	4.4	4.1
E1-Norephedrine	5.9	7.1	3.1	2.7	6.9	5.7	2.2	1.3	1.1	5.4	5.1	1.5
E2-Norephedrine	5.4	3.1	4.4	2.4	4.7	3.6	3.4	5.2	2.1	4.3	3.6	3.6
E1-PMA	2.7	8.4	5.6	1.2	8.6	6.1	2.6	0.3	0.4	5.6	5.3	1.1
E2-PMA	4.6	5.4	4.5	4.6	3.9	2.6	3.1	1.6	1.1	4.9	3.7	1.9
(+)-Ephedrine	6.9	3.5	6.5	4.3	5.7	3.2	6.3	1.0	3.4	5.6	4.4	3.6
(-)-Ephedrine and (-)-Ψephedrine	2.6	2.7	4.1	3.6	3.1	2.3	6.4	0.7	4.4	3.1	3.0	3.8
(+)-Ψephedrine	10.6	6.2	5.6	5.9	0.5	2.4	3.4	9.1	3.2	7.4	2.9	5.3

*-D indicates day

**- the following concentrations were used: 10, 100 and 1000 ng/L in the case of compounds that were not enantioseparated

Table S7 Validation parameters –ion suppression.

Analyte	Signal suppression (%) (n=4)
R-(-)-Amphetamine	38 ± 10
S-(+)-Amphetamine	53 ± 9
R-(-)-Methamphetamine	-29 ± 13
S-(+)-Methamphetamine	-6 ± 9
E1-Mephedrone	-22 ± 12
E2-Mephedrone	-40 ± 14
R-(-)-MDA	-15 ± 1
S-(+)-MDA	-13 ± 2
R-(-)-MDMA	-44 ± 7
S-(+)-MDMA	-58 ± 8
E1-MDEA	-34 ± 2
E2-MDEA	-58 ± 7
E1-HMA	-50.4 ± 6.2
E2-HMA	-69 ± 14
E1-HMMA	-82 ± 34
E2-HMMA	-77 ± 15
E1-Norephedrine	63 ± 3
E2-Norephedrine	22 ± 5
E1-PMA	-22 ± 8
E2-PMA	-39 ± 4
(+)-Ephedrine	-78 ± 5
(-)-Ephedrine and (-)-Ψephedrine	-72 ± 8
(+)-Ψephedrine	-77 ± 16

Table S8 SPE recovery for the studied analytes.

	SPE relative recovery % (n=3)		
Analyte	25 ng/L*	250 ng/L*	2500 ng/L*
R-(-)-Amphetamine	101.0 ± 6.6	76.0 ± 1.6	82.0 ± 4.7
S-(+)-Amphetamine	81.0 ± 10.6	99.0 ± 2.0	82.0 ± 4.2
R-(-)-Methamphetamine	91.0 ± 4.4	113.0 ± 0.7	82.0 ± 5.0
S-(+)-Methamphetamine	84.0 ± 1.9	86.0 ± 1.2	84.0 ± 7.1
E1-Mephedrone	109.0 ± 3.2	99.0 ± 4.8	80.0 ± 7.0
E2-Mephedrone	99.0 ± 8.5	99.0 ± 4.3	87.0 ± 11.5
R-(-)-MDA	93.0 ± 6.2	94.0 ± 4.2	81.0 ± 1.0
S-(+)-MDA	110.0 ± 8.5	99.0 ± 1.5	91.0 ± 1.5
R-(-)-MDMA	91.0 ± 3.7	81.0 ± 7.8	89.0 ± 4.3
S-(+)-MDMA	93.0 ± 1.7	100.0 ± 0.7	84.0 ± 1.9
E1-MDEA	102.0 ± 2.0	95.0 ± 8.6	91.0 ± 5.9
E2-MDEA	99.0 ± 1.8	92.0 ± 1.9	93.0 ± 13.4
E1-HMA	97.0 ± 8.7	114.0 ± 0.3	106.0 ± 16.4
E2-HMA	106.0 ± 4.6	107.0 ± 2.9	120.0 ± 11.5
E1-HMMA	84.0 ± 8.8	85.0 ± 9.4	100.0 ± 3.3
E2-HMMA	108.0 ± 7.5	105.0 ± 2.4	118.0 ± 1.7
E1-Norephedrine	112.0 ± 2.8	117.0 ± 1.1	108.0 ± 1.5
E2-Norephedrine	115.0 ± 5.9	95.0 ± 2.1	83.0 ± 1.4
E1-PMA	110.0 ± 8.5	94.0 ± 2.4	80.0 ± 0.7
E2-PMA	113.0 ± 3.5	118.0 ± 5.9	91.0 ± 0.4
(+)-Ephedrine	81.0 ± 9.0	82.0 ± 2.6	91.0 ± 2.1
(-)-Ephedrine and (-)-Ψephedrine	112.0 ± 0.6	87.0 ± 2.5	113.0 ± 9.6
(+)-Ψephedrine	104.0 ± 10.6	83.0 ± 0.3	81.0 ± 1.0

Table S9 Concentrations of analytes expressed as ng L^{-1} (n.a. means not available).

	R-(-)-Amphetamine								S-(+)-Amphetamine							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	207.5	161.0	4.0	57.6	-	161.0	33.2	144.6	153.0	138.5	13.0	48.3	-	112.1	38.1	116.0
Tues	208.5	129.5	-	205.9	-	77.9	32.3	145.7	170.5	74.0	-	165.2	-	54.3	32.4	116.6
Wed	202.0	132.8	-	172.1	-	112.6	34.8	141.9	170.5	105.7	-	138.6	-	79.3	33.5	102.4
Thur	190.5	141.3	4.0	109.6	-	118.7	33.7	171.4	147.0	101.7	6.0	91.3	-	81.6	32.2	127.2
Fri	163.5	159.8	-	134.1	-	137.2	37.4	167.4	137.5	126.2	-	111.0	-	90.6	23.0	116.3
Sat	202.5	172.9	-	173.4	-	149.4	32.3	179.0	157.5	148.6	-	146.0	-	105.5	35.8	129.8
Sun	204.5	108.5	5.0	50.3	-	182.5	33.5	180.6	147.0	103.5	6.0	43.1	-	132.1	33.2	133.5
	R-(-)-Methamphetamine								**S-(+)-Methamphetamine**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	2.0	392.9	-	<MQL	-	-	<MQL	<MQL	2.0	333.6	15.9	<MQL	<MQL	16.2	49.9	27.6
Tues	3.0	259.5	1.8	<MQL	<MQL	-	<MQL	<MQL	1.5	217.5	30.9	<MQL	<MQL	8.3	46.1	22.5
Wed	3.5	107.0	2.4	<MQL	-	-	<MQL	<MQL	2.0	92.5	23.7	<MQL	<MQL	15.7	47.1	24.0
Thur	3.0	173.1	3.7	-	-	-	<MQL	<MQL	2.0	163.4	22.0	<MQL	<MQL	13.6	40.3	18.7
Fri	-	126.5	-	-	-	-	<MQL	<MQL	-	107.0	25.9	<MQL	<MQL	16.0	46.6	20.2
Sat	8.5	217.2	-	<MQL	<MQL	-	<MQL	<MQL	2.5	178.8	28.3	<MQL	<MQL	15.5	47.1	31.4
Sun	3.5	130.2	-	<MQL	<MQL	-	<MQL	<MQL	2.0	102.8	30.1	<MQL	<MQL	14.9	44.7	25.7
	R-(-)-MDMA								**S-(+)-MDMA**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	163.0	91.9	8.9	189.1	11.0	77.6	96.0	80.0	64.5	66.1	4.5	101.6	5.0	34.6	37.0	37.0
Tues	76.5	43.7	0.0	380.4	8.1	20.0	38.0	42.0	33.5	24.3	7.4	203.7	4.7	8.7	13.0	22.0
Wed	63.0	24.6	5.0	214.7	0.0	25.7	20.0	26.0	39	18.4	3.3	107.4	0.0	13.6	7.0	16.0
Thur	44.5	42.8	1.9	74.5	0.0	22.8	14.0	28.0	28.5	14.3	1.9	54.7	0.0	12.8	8.0	18.0
Fri	71.0	23.5	6.4	226.2	8.2	26.9	22.0	33.0	51.5	13.5	3.2	154.7	5.4	15.0	14.0	22.0
Sat	265.0	60.6	21.3	353.6	7.2	63.8	57.0	120.0	184	42.9	11.6	240.6	4.8	41.6	34.0	98.0
Sun	318.0	71.2	34.1	278.1	11.8	148.6	155.0	175.0	186.5	36.3	12.0	221.2	8.2	102.7	97.0	125.0
	R-(-)-MDA								**S-(+)-MDA**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**

Mon	6.9	2.0	-	11.1	-	-	-	-	7.3	-	-	15.9	-	-	-	-
Tues	4.3	-	-	12.3	-	-	-	-	-	-	-	14.4	-	-	-	-
Wed	-	-	-	8.6	-	-	-	-	-	-	-	3.6	-	-	-	-
Thur	-	-	-	5.4	-	-	-	2.2	-	-	-	5.5	-	-	-	1.1
Fri	2.0	-	-	4.9	-	-	-	-	-	-	-	7.9	-	4.7	-	-
Sat	3.9	-	-	8.3	-	-	-	-	12.9	1.0	-	12.5	-	-	-	-
Sun	9.8	-	-	11.1	-	-	-	-	11.6	5.0	-	25.2	-	-	-	-
	E1-HMA								**E2-HMA**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	79.0	-	-	-	-	-	-	-	-	-	-	42.0	-	-	-	-
Tues	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thur	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sat	-	-	-	39.0	-	-	-	-	-	-	-	37.0	-	-	-	-
Sun	70.0	-	-	-	-	-	-	-	86.0	-	-	38.0	-	-	-	-
	E1-HMMA								**E2-HMMA**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	38.1	-	-	-	18.3	37.1	18.9	18.9	58.2	-	-	60.7	57.6	28.9	14.6	29.0
Tues	37.1	-	-	-	36.6	17.3	-	-	-	-	-	59.9	57.6	14.4	-	-
Wed	36.8	-	-	37.9	-	17.1	-	-	-	-	-	58.5	-	7.7	-	-
Thur	-	-	-	38.8	-	18.3	-	18.3	-	-	-	57.8	-	28.8	-	-
Fri	36.8	-	<MQL	42.1	-	36.7	-	15.4	47.5	-	<MQL	58.4	-	57.7	-	28.9
Sat	38.6	-	-	41.6	-	37.8	18.5	19.1	59.3	-	-	59.1	-	58.4	29.0	29.2
Sun	39.1	-	-	40.8	18.3	38.4	18.9	19.1	59.6	-	-	62.4	57.7	59.0	29.6	29.3
	R-(+)-Mephedrone								**S-(-)-Mephedrone**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	67.0	-	-	-	-	-	-	-	72.5	-	-	-	-	-	-	-
Tues	37.0	-	-	-	-	-	-	-	27.5	-	-	-	-	-	-	-
Wed	38.0	-	-	-	-	-	-	-	38	-	-	-	-	-	-	-

Thur	37.0	-	-	-	-	-	-	-	33	-	-	-	-	-	-	-
Fri	44.5	-	-	-	-	-	-	-	28.5	-	-	-	-	-	-	-
Sat	105.5	-	-	-	-	-	-	-	86	-	-	-	-	-	-	-
Sun	90.0	-	-	-	-	-	-	-	57.5	-	-	-	-	-	-	-
	E1-Norephedrine								**E2-Norephedrine**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	-	57.0	5.0	-	n.a.	n.a.	n.a.	n.a.	-	69.0	7.0	-	n.a.	n.a.	n.a.	n.a.
Tues	-	53.0	12.0	-	n.a.	n.a.	n.a.	n.a.	-	42.0	6.0	-	n.a.	n.a.	n.a.	n.a.
Wed	-	65.0	10.0	-	n.a.	n.a.	n.a.	n.a.	33.0	59.0	11.0	-	n.a.	n.a.	n.a.	n.a.
Thur	-	52.0	8.0	-	n.a.	n.a.	n.a.	n.a.	11.3	46.0	11.0	-	n.a.	n.a.	n.a.	n.a.
Fri	-	39.0	7.0	-	n.a.	n.a.	n.a.	n.a.	18.0	44.0	14.0	-	n.a.	n.a.	n.a.	n.a.
Sat	-	51.0	12.0	-	n.a.	n.a.	n.a.	n.a.	21.3	59.0	11.0	-	n.a.	n.a.	n.a.	n.a.
Sun	-	53.0	6.0	-	n.a.	n.a.	n.a.	n.a.	16.7	60.0	8.0	-	n.a.	n.a.	n.a.	n.a.
	1R,2S-(-)-Ephedrine								**1S,2S-(+)-Pseudoephedrine**							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**	**Bristol**	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Mon	-	1.0	1.0	n.a.	n.a.	n.a.	n.a.	n.a.	569.0	50.0	67.0	n.a.	n.a.	n.a.	n.a.	n.a.
Tues	8.0	-	10.0	n.a.	n.a.	n.a.	n.a.	n.a.	352.0	48.0	103.0	n.a.	n.a.	n.a.	n.a.	n.a.
Wed	1.5	-	2.0	n.a.	n.a.	n.a.	n.a.	n.a.	438.5	42.0	100.0	n.a.	n.a.	n.a.	n.a.	n.a.
Thur	-	3.0	12.0	n.a.	n.a.	n.a.	n.a.	n.a.	250.0	39.0	116.0	n.a.	n.a.	n.a.	n.a.	n.a.
Fri	1.5	3.0	-	n.a.	n.a.	n.a.	n.a.	n.a.	470.5	52.0	83.0	n.a.	n.a.	n.a.	n.a.	n.a.
Sat	5.0	0.0	39.0	n.a.	n.a.	n.a.	n.a.	n.a.	358.5	42.0	83.0	n.a.	n.a.	n.a.	n.a.	n.a.
Sun	-	3.0	-	n.a.	n.a.	n.a.	n.a.	n.a.	410.5	40.0	86.0	n.a.	n.a.	n.a.	n.a.	n.a.

Table S10 Biomarkers of drugs of abuse and precursors (AMP=amphetamine, METH=methamphetamine, MEPH=mephedrone, EPH=ephedrine, PSEUDOEPH=pseudoephedrine and NE=norephedrine). The following information are provided: parent compound or metabolite used as a DTR, urinary excretion data, CF used for WBE estimates, EF expected in urine after human metabolism (EF_{urine}), EF calculated from illegal synthesis of the drug ($EF_{illegal\_synth}$), information derived from the legal use of the drug and consumption estimates calculated from official health statistics in relation to the population served by the wastewater treatment plant in the study and from wastewater analysis.

Drug	Metabolite used as DTR	Excretion (%)	CF	EF_{Urine}	Illegal Use	Legal Use						Consumption estimates (mg day^{-1} 1000 people^{-1})	
					$EF_{illegal\_synth}$	Legally prescribed? (Y/N)	From metabolism of prescribed pharmaceuticals	Amount prescribed (kg/year)	Excretion (%)	Amount excreted as metabolite (kg/year)	$EF_{legal\ source}$	Health national system data (2015) in relation to the population served by the WWTP	Wastewater analysis (2015), calculated from weekly average loads of considered DTR
AMP	AMP	30.0% in neutral condition of pH, up to 74.0% in acidic and 1.0% in alkaline urines [39]	3.3	<0.5 as enantioselective metabolism favours *S*-(+)-AMP when (±)-AMP is consumed; *S*-(+)-AMP after dimethyl-amphetamine intake (excretion 0.65%) [3]	0.5 (when Leuckart method) or >0.5 (when reduction of diastereoisomers of NE and norpseudoephedrine-less common)	Y	73.4% *S*-(+)-AMP base from dexamphetamine sulfate	23.67 kg/year as dexamfetamine sulfate in England (or 17.38 kg/year as *S*-(+)-AMP)	30.0% in neutral condition of pH, up to 74.0% in acidic and 1.0% in alkaline urines [39]	5.21 kg in neutral urine pH in England (or 12.86 kg in case of acidic urine or 0.17 kg in case of alkaline urine)	In general 0.5 and >0.5 [17]; >0.5 as dexampheta mine sulfate	0.32 as *S*-(+)-AMP in Bristol	272.7 as (±)-AMP, of which 120.1 as *S*-(+)-AMP in Bristol; 213.7 as NE in Bristol
	NE	2.0% in neutral condition of pH [39]	44.7				Amphetaminil	No in England	Low in urine [40], 3.3% [20]				
							Benzphetamine	No in England	Minor [41], 7% under acidic conditions, 2% in alkaline [19] only *S*-(+)-enantiomer		>0.5		
							Clobenzorex	No in England	5% of (±)-AMP [17]				
							Ethylamphetamine	No in England	10.7±1.3% of *S*-(+)-AMP (n=2), 4.8±0.6% of *R*-(-)-AMP (n=2) [42]		<0.5		
							Famprofazone	No in England	(±)-AMP at the beginning of the		0.5 at the beginning of		

									administration and then *R*-(-)-enantiomer increases [43]		the administration, then <0.5		
							Fencamine	No in England	(±)-AMPH [18]		0.5		
							Fenethylline	No in England	24.5 % [44],26.7% [45] of (±)-AMP [17]		0.5		
							Fenproporex	No in England	27-34% (±)-AMP [46]		0.5		
							Furfenorex	No in England	4.1% AMP [20]				
							Lisdexamfetamine	68.35 kg/year as lisdexamfetamine dimesylate in England (or 20.30 kg/year as *S*-(+)-AMP)	41.5% *S*-(+)-AMP [47]	8.42 kg in England	>0.5	1.26 as *S*-(+)-AMP in Bristol	272.7 as (±)-AMP, of which 120.1 as *S*-(+)-AMPH in Bristol; 213.7 as NE in Bristol
							Mefenorex	No in England	5.5% after 40 mg and 10.4% after 80 mg [48]				
							Mesocarb	No in England	4%				
							Prenylamine	No in England	higher amounts of *S*-(+)-AMP initially [17]		>0.5		
							Selegiline	9.72 kg/year in England	3.06±1.10 (n=4) as *R*-(-)-AMP [49], 13.5% [20]	1.31 kg as *R*-(-)-AMP in England (using 13.5% as E)	<0.5	0.10 as *R*-(-)-AMP in Bristol	272.7 as (±)-AMP, of which 152.6 as *R*-(-)-AMP in Bristol; 213.7 as NE in Bristol
METH	METH	43.0% at pH range between 6 and 8, up to 76.0% in acidic and 2.0% in alkaline urines [39]	2.3	<0.5 (because metabolism favours S-(+)-enantiomer) when (±)-METH is consumed [14]; *S*-(+)-METH after dimethyl-amphetami	0.5 (when Leuckart route and reductive amination) or >0.5 (when (1R,2S)-(-)-EPH or (1S,2S)-(+)-PSEUDOEPH)	Y	METH	No in England			>0.5 as the form of all pharmaceutical METH is the *S*-(+)-enantiomer [17]		

				ne intake (excretion 7.5%) [3]									
	AMP	4.0-7.0% at pH range between 6 and 8 [39]	20.1				Benzphetamine	No in England	Minor [41], ~2% [19] only *S*-(+)--enantiomer		>0.5		
	NE	5% [33]	19.7	(1*R*,2*S*)-(-)-NE derived from *S*-(+)-AMP of the METH metabolism [34]			Famprofazone	No in England	30% S-(+)-METH and 70% R-(-)-enantiomer [45]		<0.5		
							Fencamine	No in England	(±)-METH [18]		0.5		
							Furfenorex	No in England	3.7% METH [20]				
							Mefenorex	No in England	(±)-METH		0.5		
							Selegiline	9.72 kg/year in England	36.96±8.17 (n=4) as *R*-(-)-METH [49]; 27.5% [20]	2.67 kg as *R*-(-)-METH in England	<0.5	0.18 as *R*-(-)-METH in Bristol	2.7 as (±)-METH, of which 1.8 as *R*-(-)-METH in Bristol; 1661.1 as (±)-AMP and 94.2 as NE in Bristol
MEPH	MEPH	15.4±8.4 %(n=6, dose=150 mg MEPH) [50]	6.5		0.5 [32]	N							166.4 as (±)-MEPH in Bristol
MDA	MDA	Unchanged (overdose case)	n.a.	<0.5 (because metabolism favours	0.5	N							No CF available

		[39]		S-(+)-MDA)								
MDMA	MDMA	65% [51], 15.0% [52]	1.5 (6.7 when 15% was applied)	<0.5 (because metabolism favours S-(+)-MDMA, also faster eliminated [24, 25])	0.5 (when Leuckart method or reductive amination reactions) [22, 23]	N						As (±)-MDMA: 56.5 in Oslo, 79.7 in Bristol, 48.1 in Lyngby, 93.0 in Utrecht, 32.9 in Brussels, 65.2 in Zurich, 10.3 in Milan and 3.5 in Castellon
	MDA	1.5% [52]	71.9	>0.5 after (±)-MDMA intake [23-25]								As (±)-MDA: 36.0 in Oslo, 140.7 in Bristol, 9.2 in Lyngby, 232.1 in Utrecht, 11.3 in Brussels, 0.0 in Zurich, Milan and Castellon
	HMMA	20.0% [52]	5.0	>0.5, <0.5 as HMMA sulphate								As (±)-HMMA: 0.0 in Oslo, 77.3 in Bristol, 39.6 in Lyngby, 68.8 in Utrecht, 83.0 in Brussels, 47.4 in Zurich, 0.0 in Milan and 37.6 in Castellon
	HMA	1.0% [52]	106.6									As (±)-HMA: 0.0 in Oslo, 785.8 in Bristol, 0.0 in Lyngby, 365.5 in Utrecht, 0.0 in Brussels, Zurich, Milan and Castellon
MDEA	MDEA	19.0%±2.55 [53]	5.3	<0.5 (because metabolism favours S-(+)-MDEA) [54]	0.5	N						Not found
	MDA	28.0% [39]	4.1	>0.5 after (±)-MDEA intake								
PMA	PMA	15% [55]	6.7		0.5 (Leuckart method) [56]	N						Not found
EPH	EPH	70.0-80.0% [39]	1.3			Y, in the form of 1*R*,2*S*-	EPH	0.83 kg/year as EPH hydrochloride in England (or	75% used as average	0.46 kg in England	0.03 as EPH in Bristol	0.8 in Bristol, 0.9 in Oslo, 4.4 in Italy as 1*R*,2*S*-(−)-EPH

						(−)-EPH		0.62 kg/year as EPH)				
	NE	4.0% [39] 8-20% as NE, of which 4% is further metabolised to 4-hydroxy-NE and hippuric acid [57]	27.3				Famprofazone	No in England	Higher (-)-EPH [49]			93.2 in Bristol, 1391.1 in Oslo, 193.8 in Italy as (±)-NE
							Selegiline	9.72 kg/year in England	0.62±0.29 (n=4) as (1*S*,2*R*)-(+)-EPH [49]	0.06 kg as (1*S*,2*R*)-(+)-EPH in England	0.004 as (1*S*,2*R*)-(+)-EPH in Bristol	
PSEUDOEPH	PSEUDOEPH	88.0% [39]	1.1			Y in the form of 1*S*,*2*S-(+)-PSEUDOEPH	Famprofazone	No in England	Higher (-)-PSEUDOEPH			106.0 in Bristol, 23.3 in Oslo, 39.3 in Italy as 1*S*,*2*S-(+)-PSEUDOEPH
							PSEUDOEPH	253.54 kg/year as PSEUDOEPH hydrochloride in England (or 223.12 kg/year as 1*S*,2S-(+)-PSEUDOEPH)	88.0% [39]	196.34 kg as 1*S*,2S-(+)-PSEUDOEPH in England	10.61 as 1*S*,2S-(+)-PSEUDOEPH in Bristol	
							Selegiline	9.72 kg/year in England	0.04±0.03 (n=4) as (1*R*,2*R*)-(-)-PSEUDOEPH [49]	0.004 kg as (1*R*,2*R*)-(-)-PSEUDOEPH in England	0.0002 as (1*R*,2*R*)-(-)-PSEUDOEPH in Bristol	
NE	NE	86.3%[58]	1.2			Y as oral decongestant [59]	Dexamfetamine	23.67 kg/year as dexamfetamine sulfate in England	2.0% in neutral condition of urine pH [39]	0.35 kg in neutral urine pH in England	0.02 as NE in Bristol	4.1 in Bristol, 61.2 in Oslo, 8.5 in Italy as (±)-NE
							EPH	0.83 kg/year as EPH hydrochloride in England	4.0% [39]	0.02 kg in England	0.0015 as NE in Bristol	
							Famprofazone	No in England	Higher (-)-NE [60]			
							Selegiline	9.72 kg/year in England	0.12±0.05 (n=4) as (1*S*,2*R*)-NE [49]	0.011 kg as (1*S*,2*R*)-NE in England	0.0008 as (1*S*,2*R*)-NE in Bristol	

S1. Amphetamine

Amphetamine has one chiral centre and exists in two enantiomeric forms [1]. Racemic amphetamine undergoes enantioselective metabolism by favouring *S*-(+)-enantiomer [2] and leading to enrichment of the *R*-(-)-amphetamine when excreted in urine (EF_{urine}<0.5). However, in the case of *S*-(+)-dimethyl-amphetamine consumption, EF_{urine} will be >0.5 as only *S*-(+)-amphetamine is produced [3]. Synthesis of (±)-amphetamine is commonly performed via Leuckart method, which uses 1-phenyl-2-propanone, formic acid, ammonium formate or formamide as reagents [4]. A stereoselective method, which involves the reduction of appropriate diastereoisomers of norephedrine or norpseudoephedrine [5], is much less common [6]. Therefore, expected $EFs_{illegal\_synth}$ are 0.5 and >0.5, respectively, depending on the synthesis route. In Europe, licit amphetamine is prescribed as enantiopure *S*-(+)-amphetamine (e.g. dexamfetamine sulphate known as Dexamed in the UK and in Denmark, Attentin in Norway, Amfexa in Spain and Tentin in the Netherlands [7]) and as racemate in other prescription drugs only under the Medicines Act. The prodrug lisdexamfetamine (as dimesylate salt) is completely metabolised to *S*-(+)-amphetamine and it is also available in the European market [8, 9] (e.g. trade name Elvanse in the UK and in Denmark and authorised in Spain [10]). Pharmaceuticals, such as fenproporex [11] and clobenzorex [12], are metabolised to (±)-amphetamine (Table S9). If excreted as a metabolite of *R*-(-)-selegiline it is enriched in the *R*-(-)-amphetamine along with *R*-(-)-methamphetamine [13].

S2. Methamphetamine

Similarly to amphetamine, methamphetamine undergoes stereoselective metabolism in humans by favouring the *S*-(+)-enantiomer [14] and leading to the enrichment of *R*-(-)-enantiomer in urine with a changing enantiomeric ratio over the time, resulting in EF_{urine}<0.5. Illicit methamphetamine is synthesised by two major routes. The first starts from the reactions of phenylacetone (i.e. the Leuckart route and reductive amination) leading to racemic methamphetamine, whilst the other starts from (*1R,2S*)-(-)-ephedrine [or (*1S,2S*)-(+)-pseudoephedrine] that is reduced with red phosphorus and hydroiodic acid stereoselectively synthesising the more potent *S*-(+)-isomer. As a consequence, the expected $EFs_{illegal\_synth}$ are 0.5 and >0.5, respectively.

The EMCDDA reports indicate that the production of enantio-enriched methamphetamine from ephedrine and pseudoephedrine is commonly used in Central Europe, whilst the synthesis with phenylacetone as the precursor is preferred in Lithuania [15, 16]. Both synthetic routes have been confirmed to be used in clandestine laboratories in the Netherlands [16]. As the form of all pharmaceutical methamphetamine is the *S*-(+)-enantiomer [17], $EF_{legal\ source}$ is >0.5 in the case of disposal. Controlled pharmaceuticals, such as mefenorex and fencamine [18], produce (±)-methamphetamine as a metabolite, whilst others, such as famprofazone, are converted to 30% *S*-(+)-methamphetamine and 70% *R*-(-)-enantiomer [14]. The metabolism of benzphetamine leads to only ~2% of *S*-(+)-methamphetamine [19], whilst the metabolism of selegiline results in 28% of *R*-(-)-methamphetamine [20]. Therefore, if (±)-methamphetamine is consumed, it will be found in urine enriched with the *R*-(-)-enantiomer and, additionally, generated amphetamine will be enriched with the *S*-(+)-enantiomer. If enantiopure *S*-(+)-methamphetamine is consumed, it will be excreted in urine as enantiopure *S*-(+)-methamphetamine and *S*-(+)-amphetamine. This is because chiral inversion does not take place during human metabolism.

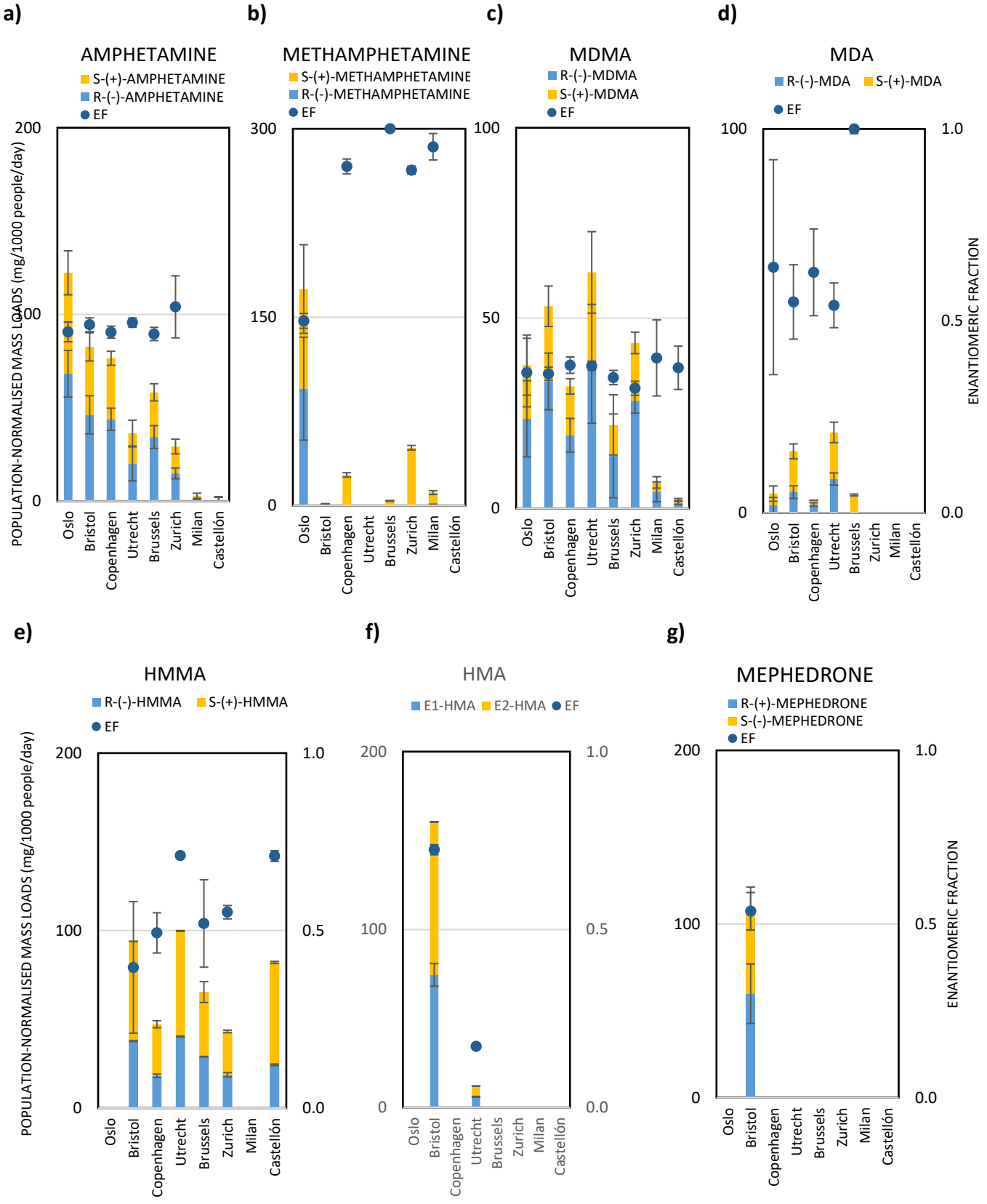

Figure S2 Average population-normalised mass loads and average enantiomeric fraction values in a week monitoring campaign. For HMMA: EF values are reported assuming that the first-eluting enantiomer is *R*-(-)-HMMA and the second one is *S*-(+)-HMMA. Due to high standard deviation values for amphetamine in Milan, an average EF value is not displayed.

Table S11 Amphetamine loads. LOADS are population-normalised mass loads, expressed as mg/1000 people/day, and CONS is estimated consumption.

R-(-)-Amphetamine																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	46.2	152.5	70.6	233.0	1.1	3.6	8.8	29.1	-	-	39.6	130.7	14.3	47.3	40.5	133.6
Tues	48.1	158.7	56.4	186.0	-	-	30.6	100.9	-	-	29.4	97.0	12.7	41.8	41.4	136.7
Wed	45.3	149.6	76.3	251.7	-	-	27.4	90.4	-	-	27.6	91.2	13.3	44.0	39.3	129.8
Thur	42.4	140.0	75.0	247.6	0.7	2.5	16.5	54.3	-	-	29.3	96.6	13.2	43.7	46.8	154.3
Fri	46.6	153.8	76.3	251.9	-	-	22.1	73.1	-	-	33.8	111.4	14.7	48.6	45.8	151.1
Sat	50.4	166.3	76.4	252.3	-	-	26.6	87.8	-	-	36.5	120.5	15.8	52.0	46.4	153.3
Sun	44.6	147.0	46.8	154.4	1.0	3.2	7.9	25.9	-	-	44.1	145.4	19.9	65.5	46.7	154.0
AV	46.2	152.6	68.3	225.3	0.9	3.1	20.0	65.9	-	-	34.3	113.3	14.8	49.0	43.8	144.7
SD	2.5	8.4	11.9	39.3	0.2	0.6	9.1	30.1	-	-	6.1	20.1	2.4	8.1	3.3	10.8
CV	0.06	0.06	0.17	0.17	0.18	0.18	0.46	0.46	-	-	0.18	0.18	0.16	0.16	0.07	0.07
S-(+)-Amphetamine																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	34.1	112.5	60.7	200.4	3.5	11.7	7.4	24.4	-	-	27.6	91.0	16.5	54.3	32.5	107.2
Tue	39.3	129.8	32.2	106.3	-	-	24.5	81.0	-	-	20.5	67.6	12.7	42.0	33.1	109.4
Wed	38.3	126.2	60.7	200.3	-	-	22.0	72.8	-	-	19.5	64.3	12.8	42.4	28.4	93.7
Thur	32.7	108.1	54.0	178.1	1.1	3.7	13.7	45.2	-	-	20.1	66.5	12.6	41.7	34.7	114.5
Fri	39.2	129.3	60.3	199.1	-	-	18.3	60.5	-	-	22.3	73.6	9.1	29.9	31.8	104.9
Sat	39.2	129.4	65.7	216.9	-	-	22.4	73.9	-	-	25.8	85.0	17.5	57.6	33.7	111.2
Sun	32.0	105.7	44.6	147.3	1.2	3.8	6.7	22.2	-	-	31.9	105.3	19.7	64.9	34.5	113.9
AV	36.4	120.1	54.0	178.3	1.9	6.4	16.5	54.3	-	-	24.0	79.0	14.4	47.5	32.7	107.8
SD	3.3	10.9	11.8	38.8	1.4	4.6	7.3	24.1	-	-	4.6	15.3	3.6	11.9	2.2	7.1
CV	0.09	0.09	0.22	0.22	0.71	0.71	0.44	0.44	-	-	0.19	0.19	0.25	0.25	0.07	0.07
(±)-Amphetamine																

	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	80.3	265.0	131.3	433.3	4.6	15.2	16.2	53.6	-	-	67.2	221.8	30.8	101.7	73.0	240.9
Tue	87.4	288.5	88.6	292.3	-	-	55.1	181.9	-	-	49.9	164.5	25.4	83.8	74.6	246.0
Wed	83.6	275.8	137.0	452.0	-	-	49.4	163.1	-	-	47.1	155.5	26.2	86.4	67.7	223.4
Thur	75.2	248.1	129.0	425.8	1.9	6.2	30.2	99.5	-	-	49.4	163.1	25.9	85.4	81.4	268.8
Fri	85.8	283.1	136.7	451.0	-	-	40.5	133.6	-	-	56.1	185.1	23.8	78.5	77.6	256.0
Sat	89.6	295.7	142.2	469.2	-	-	49.0	161.8	-	-	62.3	205.5	33.2	109.6	80.1	264.4
Sun	76.6	252.7	91.4	301.7	2.1	7.0	14.6	48.1	-	-	76.0	250.7	39.5	130.5	81.2	267.9
AV	82.6	272.7	122.3	403.6	2.9	9.5	36.4	120.2	-	-	58.3	192.3	29.3	96.5	76.5	252.5
SD	5.5	18.1	22.5	74.2	1.5	5.0	16.4	54.2	-	-	10.7	35.3	5.6	18.6	5.1	16.7
CV	0.07	0.07	0.18	0.18	0.53	0.53	0.45	0.45	-	-	0.18	0.18	0.19	0.19	0.07	0.07

Table S12 Methamphetamine loads. LOADS are population-normalised mass loads, expressed as mg/1000 people/day, and CONS is estimated consumption.

R-(-)-Methamphetamine																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	0.4	1.0	172.3	396.2	0.0	0.0	-	-	-	-	-	-	-	-	-	-
Tue	0.7	1.6	113.0	259.8	0.7	1.6	-	-	-	-	-	-	-	-	-	-
Wed	0.8	1.8	61.4	141.3	0.9	2.0	-	-	-	-	-	-	-	-	-	-
Thur	0.7	1.5	91.9	211.3	1.4	3.2	-	-	-	-	-	-	-	-	-	-
Fri	0.0	0.0	60.4	139.0	0.0	0.0	-	-	-	-	-	-	-	-	-	-
Sat	2.1	4.9	96.1	220.9	0.0	0.0	-	-	-	-	-	-	-	-	-	-
Sun	0.8	1.8	56.1	129.1	0.0	0.0	-	-	-	-	-	-	-	-	-	-
AV	0.8	1.8	93.0	214.0	0.4	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SD	0.6	1.5	41.0	94.4	0.6	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CV	0.83	0.83	0.44	0.44	1.34	1.34	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-(+)-Methamphetamine																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOAD	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	0.4	1.0	146.3	336.4	8.6	19.9	-	-	-	-	4.0	9.2	21.6	49.6	7.7	17.8
Tue	0.3	0.8	94.7	217.7	11.9	27.3	-	-	-	-	3.1	7.2	18.1	41.6	6.4	14.7
Wed	0.4	1.0	53.1	122.2	8.7	20.0	-	-	-	-	3.9	8.9	18.0	41.5	6.7	15.3
Thur	0.4	1.0	86.8	199.6	8.3	19.0	-	-	-	-	3.3	7.7	15.8	36.4	5.1	11.7
Fri	0.0	0.0	51.1	117.6	9.5	21.8	-	-	-	-	3.9	9.1	18.3	42.2	5.5	12.7
Sat	0.6	1.4	79.1	181.8	10.4	23.9	-	-	-	-	3.8	8.7	23.0	52.8	8.1	18.7
Sun	0.4	1.0	44.3	102.0	11.6	26.6	-	-	-	-	3.6	8.3	26.5	60.9	6.6	15.3
AV	0.4	0.9	79.3	182.5	9.8	22.6	0.0	0.0	0.0	0.0	3.7	8.4	20.2	46.4	6.6	15.2
SD	0.2	0.4	35.3	81.2	1.5	3.4	0.0	0.0	0.0	0.0	0.3	0.7	3.7	8.4	1.1	2.5
CV	0.49	0.49	0.44	0.44	0.15	0.15	0.0	0.0	0.0	0.0	0.09	0.09	0.18	0.18	0.17	0.17

(±)-Methamphetamine																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	0.9	2.0	318.5	732.6	8.6	19.9	-	-	-	-	4.0	9.2	21.6	49.6	7.7	17.8
Tue	1.0	2.4	207.6	477.5	12.6	28.9	-	-	-	-	3.1	7.2	18.1	41.6	6.4	14.7
Wed	1.2	2.8	114.6	263.5	9.5	22.0	-	-	-	-	3.9	8.9	18.0	41.5	6.7	15.3
Thur	1.1	2.6	178.7	410.9	9.6	22.2	-	-	-	-	3.3	7.7	15.8	36.4	5.1	11.7
Fri	0.0	0.0	111.6	256.6	9.5	21.8	-	-	-	-	3.9	9.1	18.3	42.2	5.5	12.7
Sat	2.7	6.3	175.1	402.8	10.4	23.9	-	-	-	-	3.8	8.7	23.0	52.8	8.1	18.7
Sun	1.2	2.8	100.5	231.1	11.6	26.6	-	-	-	-	3.6	8.3	26.5	60.9	6.6	15.3
AV	1.2	2.7	172.4	396.4	10.3	23.6	0.0	0.0	0.0	0.0	3.7	8.4	20.2	46.4	6.6	15.2
SD	0.8	1.9	76.2	175.3	1.4	3.2	0.0	0.0	0.0	0.0	0.3	0.7	3.7	8.4	1.1	2.5
CV	0.69	0.69	0.44	0.44	0.13	0.13	0.0	0.0	0.0	0.0	0.09	0.09	0.18	0.18	0.17	0.17

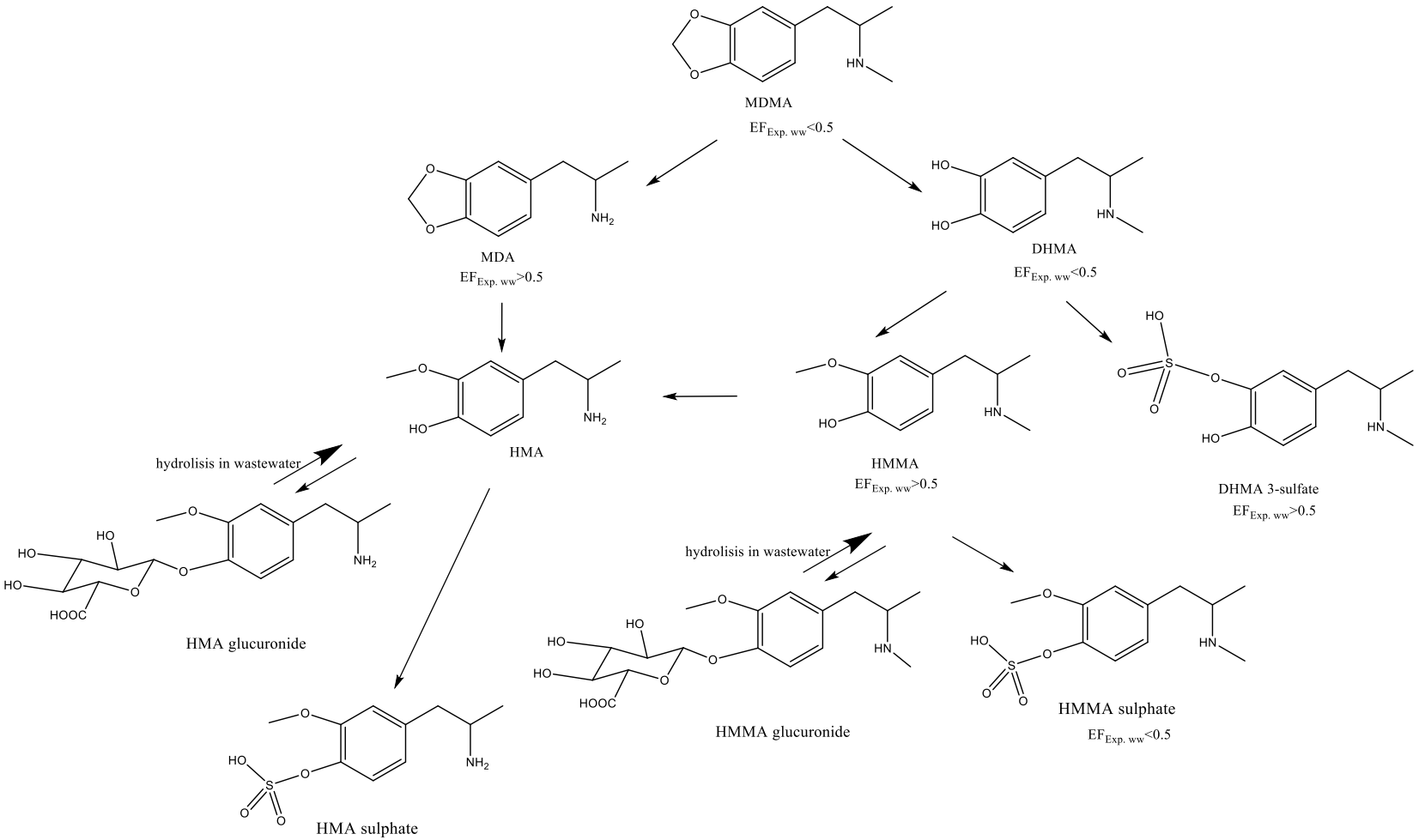

Figure S3 Expected EF values in wastewater for MDMA consumption using the analytical conditions described in Castrignanò et al. DHMA, DHMA sulphate, HMMA glucuronide and HMMA sulphate were never detected in wastewater. The hypothesis is that HMMA glucuronide is hydrolysed by bacteria, giving HMMA enriched of the S-enantiomer.

S3. MDMA

MDMA has one chiral centre and as a result it exists in two enantiomeric forms [21]. Synthesis of MDMA utilises non-stereoselective synthetic pathways [22] [23]. MDMA is therefore illegally distributed as racemate ($EF_{illegal\_synth}$=0.5). Consequently, if directly disposed of, it will be quantified as a racemate in wastewater. Furthermore, MDMA is stereoselectively metabolised with preferential metabolism of *S*-(+)-MDMA (EF_{urine}=0.43 after a fatal poisoning [24]; EF_{urine}=0.30±0.00 from urinary recovery of MDMA enantiomers after oral administration of (±)-MDMA between 0-24 h in n=8 [25]), which is also eliminated faster than *R*-(-)-MDMA [24], and formation of MDA enriched with *S*-(+)-enantiomer (EF_{urine}=0.69 after a fatal poisoning [54]; EF_{urine}=0.58±0.02 from urinary recovery of MDA enantiomers after oral administration of (±)-MDMA [25]) [23]. Hence, if found in wastewater after consumption, MDMA will be enriched with *R*-(-)-enantiomer.

Table S13 MDMA loads. LOADS are population-normalised mass loads, expressed as mg/1000 people/day, and CONS is estimated consumption.

R-(-)-MDMA																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	36.3	54.5	40.3	60.5	4.9	7.3	29.0	43.5	2.3	3.4	19.1	28.6	41.5	62.2	22.4	33.6
Tue	17.6	26.5	19.0	28.5	0.0	0.0	56.5	84.8	1.8	2.7	7.6	11.3	14.9	22.4	11.9	17.9
Wed	14.1	21.2	14.1	21.2	1.8	2.7	34.2	51.3	0.0	0.0	6.3	9.5	7.7	11.5	7.2	10.8
Thur	9.9	14.9	22.7	34.0	0.7	1.1	11.2	16.8	0.0	0.0	5.6	8.5	5.5	8.2	7.6	11.5
Fri	20.2	30.4	11.3	16.9	2.3	3.5	37.3	56.0	2.0	3.0	6.6	10.0	8.7	13.0	9.0	13.5
Sat	66.0	98.9	26.8	40.2	7.8	11.7	54.3	81.4	1.5	2.3	15.6	23.4	27.8	41.7	31.1	46.7
Sun	69.3	103.9	30.7	46.0	13.1	19.7	43.5	65.2	2.4	3.7	35.9	53.8	91.9	137.8	45.2	67.8
AV	33.4	50.0	23.6	35.3	4.4	6.6	38.0	57.0	2.0	2.2	13.8	20.7	28.3	42.4	19.2	28.8
SD	24.8	37.3	10.0	15.0	4.7	7.0	15.6	23.3	0.4	1.5	11.0	16.6	30.9	46.3	14.5	21.8
CV	0.7	0.7	0.43	0.43	1.07	1.07	0.41	0.41	0.18	0.71	0.80	0.80	1.1	1.1	0.8	0.8
S-(+)-MDMA																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	14.4	21.6	29.0	43.5	2.4	3.6	15.6	23.4	1.0	1.6	8.5	12.8	16.0	24.0	10.4	15.5
Tue	7.7	11.6	10.6	15.9	2.8	4.3	30.3	45.4	1.0	1.6	3.3	4.9	5.1	7.7	6.3	9.4
Wed	8.8	13.1	10.6	15.9	1.2	1.8	17.1	25.6	0.0	0.0	3.3	5.0	2.7	4.0	4.4	6.7
Thur	6.3	9.5	7.6	11.3	0.7	1.1	8.2	12.3	0.0	0.0	3.2	4.8	3.1	4.7	4.9	7.4
Fri	14.7	22.0	6.4	9.6	1.2	1.7	25.5	38.3	1.3	2.0	3.7	5.5	5.5	8.3	6.0	9.0
Sat	45.8	68.7	19.0	28.5	4.3	6.4	36.9	55.4	1.0	1.5	10.2	15.2	16.6	24.9	25.4	38.1
Sun	40.6	61.0	15.7	23.5	4.6	6.9	34.6	51.9	1.7	2.5	24.8	37.2	57.5	86.2	32.3	48.5
AV	19.8	29.6	14.1	21.2	2.5	3.7	24.0	36.0	1.2	1.3	8.1	12.2	15.2	22.8	12.8	19.2
SD	16.4	24.6	7.9	11.8	1.5	2.3	10.7	16.1	0.3	1.0	7.9	11.8	19.5	29.3	11.3	17.0
CV	0.8	0.8	0.56	0.56	0.63	0.63	0.45	0.45	0.24	0.73	0.97	0.97	1.3	1.3	0.9	0.9

(±)-MDMA																
	Bristol		**Oslo**		**Milan**		**Utrecht**		**Castellón**		**Brussels**		**Zurich**		**Copenhagen**	
	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS	LOADS	CONS
Mon	50.7	76.0	69.3	103.9	7.3	10.9	44.5	66.8	3.3	5.0	27.6	41.4	57.5	86.2	32.8	49.2
Tue	25.4	38.1	29.6	44.4	2.8	4.3	86.8	130.2	2.9	4.3	10.8	16.3	20.0	30.0	18.2	27.3
Wed	22.9	34.3	24.7	37.0	3.0	4.6	51.3	76.9	0.0	0.0	9.7	14.5	10.3	15.5	11.6	17.5
Thur	16.3	24.4	30.3	45.4	1.4	2.1	19.4	29.1	0.0	0.0	8.8	13.2	8.6	13.0	12.5	18.8
Fri	34.9	52.4	17.7	26.5	3.5	5.2	62.9	94.3	3.3	4.9	10.3	15.5	14.2	21.3	15.0	22.6
Sat	111.8	167.6	45.8	68.7	12.0	18.1	91.2	136.8	2.5	3.8	25.7	38.6	44.4	66.6	56.6	84.9
Sun	109.9	164.9	46.4	69.5	17.7	26.6	78.1	117.1	4.1	6.2	60.7	91.0	149.4	224.0	77.5	116.3
AV	53.1	79.7	37.7	56.5	6.8	10.3	62.0	93.0	3.2	3.5	21.9	32.9	43.5	65.2	32.0	48.1
SD	40.9	61.4	17.5	26.2	6.0	9.0	25.7	38.5	0.6	2.5	18.8	28.3	50.2	75.3	25.7	38.5
CV	0.8	0.8	0.46	0.46	0.88	0.88	0.41	0.41	0.18	0.71	0.86	0.86	1.2	1.2	0.8	0.8

Table S14 MDA loads.

R-(-)-MDA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	1.5	0.9	-	1.7	-	-	-	-
Tuesday	1.0	-	-	1.8	-	-	-	-
Wednesday	-	-	-	1.4	-	-	-	-
Thursday	-	-	-	0.8	-	-	-	0.6
Friday	0.6	-	-	0.8	-	-	-	-
Saturday	1.0	-	-	1.3	-	-	-	-
Sunday	2.1	-	-	1.7	-	-	-	-
AV	0.9	0.1	0.0	1.4	0.0	0.0	0.0	0.0
SD	0.8	0.3	0.0	0.4	0.0	0.0	0.0	0.0
CV	0.9	2.6	0.0	0.3	0.0	0.0	0.0	0.0
S-(+)-MDA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	1.6	-	-	2.4	-	-	-	-
Tuesday	-	-	-	2.1	-	-	-	-
Wednesday	-	-	-	0.6	-	-	-	-
Thursday	-	-	-	0.8	-	-	-	0.3
Friday	-	-	-	1.3	-	1.1	-	-
Saturday	3.2	0.4	-	1.9	-	-	-	-
Sunday	2.5	2.2	-	3.9	-	-	-	-
AV	1.1	0.4	0.0	1.9	0.0	0.0	0.0	0.0
SD	1.4	0.8	0.0	1.1	0.0	0.0	0.0	0.0
CV	1.3	2.2	0.0	0.6	0.0	0.0	0.0	0.0
(±)-MDA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	3.2	0.9	-	4.1	-	-	-	-
Tuesday	1.0	-	-	4.0	-	-	-	-
Wednesday	-	-	-	1.9	-	-	-	-
Thursday	-	-	-	1.6	-	-	-	0.9
Friday	0.6	-	-	2.1	-	1.1	-	-
Saturday	4.2	0.4	-	3.2	-	-	-	-
Sunday	4.7	2.2	-	5.7	-	-	-	-
AV	1.9	0.5	0.0	3.2	0.0	0.0	0.0	0.0
SD	2.0	0.8	0.0	1.5	0.0	0.0	0.0	0.0
CV	1.0	1.6	0.0	0.5	0.0	0.0	0.0	0.0

Table S15 HMA loads.

E1-HMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	17.6	-	-	-	-	-	-	-
Tuesday	-	-	-	-	-	-	-	-
Wednesday	-	-	-	-	-	-	-	-
Thursday	-	-	-	-	-	-	-	-
Friday	-	-	-	-	-	-	-	-
Saturday	-	-	-	6.0	-	-	-	-
Sunday	15.3	-	-	-	-	-	-	-
AV	4.7	0.1	0.0	0.9	0.0	0.0	0.0	0.0
SD	8.0	0.3	0.0	2.3	0.0	0.0	0.0	0.0
CV	1.7	2.6	0.0	2.6	0.0	0.0	0.0	0.0
E2-HMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	-	-	-	6.4	-	-	-	-
Tuesday	-	-	-	-	-	-	-	-
Wednesday	-	-	-	-	-	-	-	-
Thursday	-	-	-	-	-	-	-	-
Friday	-	-	-	-	-	-	-	-
Saturday	-	-	-	5.7	-	-	-	-
Sunday	18.7	-	-	5.9	-	-	-	-
AV	2.7	0.1	0.0	2.6	0.0	0.0	0.0	0.0
SD	7.1	0.2	0.0	3.2	0.0	0.0	0.0	0.0
CV	2.6	1.8	0.0	1.3	0.0	0.0	0.0	0.0
(±)-HMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	17.6	-	-	6.4	-	-	-	-
Tuesday	-	-	-	-	-	-	-	-
Wednesday	-	-	-	-	-	-	-	-
Thursday	-	-	-	-	-	-	-	-
Friday	-	-	-	-	-	-	-	-
Saturday	-	-	-	11.7	-	-	-	-
Sunday	34.0	-	-	5.9	-	-	-	-
AV	7.4	0.2	0.0	3.4	0.0	0.0	0.0	0.0
SD	13.4	0.3	0.0	4.7	0.0	0.0	0.0	0.0
CV	1.8	1.5	0.0	1.4	0.0	0.0	0.0	0.0

Table S16 HMMA loads.

E1-HMMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	8.5	-	-	-	3.8	9.1	8.2	5.3
Tuesday	8.6	-	-	-	8.2	6.5	-	-
Wednesday	8.3	-	-	6.0	-	4.2	-	-
Thursday	-	-	-	5.8	-	4.5	-	5.0
Friday	10.5	-	-	7.0	-	9.0	-	4.2
Saturday	9.6	-	-	6.4	-	9.2	9.0	4.9
Sunday	8.5	-	-	6.4	3.8	9.3	11.2	4.9
AV	7.7	0.1	0.0	4.5	2.3	7.4	4.1	3.5
SD	3.5	0.3	0.0	3.1	3.2	2.3	5.1	2.4
CV	0.5	2.6	0.0	0.7	1.4	0.3	1.3	0.7
E2-HMMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	13.0	-	-	9.3	12.0	7.1	6.3	8.1
Tuesday	-	-	-	8.9	12.9	5.4	-	-
Wednesday	-	-	-	9.3	-	1.9	-	-
Thursday	-	-	-	8.7	-	7.1	-	-
Friday	13.5	-	-	9.6	-	14.2	-	7.9
Saturday	14.8	-	-	9.1	-	14.3	14.2	7.6
Sunday	13.0	-	-	9.8	11.9	14.2	17.5	7.6
AV	7.8	0.1	0.0	9.2	5.3	9.2	5.4	4.4
SD	7.3	0.2	0.0	0.4	6.6	5.0	7.5	4.2
CV	0.9	1.8	0.0	0.0	1.2	0.5	1.4	0.9
(±)-HMMA								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)							
	Bristol	**Oslo**	**Milan**	**Utrecht**	**Castellón**	**Brussels**	**Zurich**	**Copenhagen**
Monday	21.4	-	-	9.3	15.8	16.3	14.5	13.4
Tuesday	8.6	-	-	8.9	21.1	12.0	-	-
Wednesday	8.3	-	-	15.4	-	6.1	-	-
Thursday	-	-	-	14.5	-	11.6	-	5.0
Friday	24.0	-	-	16.6	-	23.2	-	12.1
Saturday	24.4	-	-	15.5	-	23.5	23.2	12.5
Sunday	21.5	-	-	16.1	15.7	23.5	28.7	12.5
AV	15.5	0.2	0.0	13.8	7.5	16.6	9.5	7.9
SD	9.7	0.3	0.0	3.2	9.5	7.0	12.5	6.1
CV	0.6	1.5	0.0	0.2	1.3	0.4	1.3	0.8

Table S17 Mephedrone loads.

R-(+)-Mephedrone									
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)								ESTIMATED CONSUMPTION
	Bristol	Oslo	Milan	Utrecht	Castellón	Brussels	Zurich	Copenhagen	UK (Bristol)
Monday	14.9	-	-	-	-	-	-	-	96.9
Tuesday	8.5	-	-	-	-	-	-	-	55.3
Wednesday	8.5	-	-	-	-	-	-	-	55.3
Thursday	8.2	-	-	-	-	-	-	-	53.3
Friday	12.7	-	-	-	-	-	-	-	82.6
Saturday	26.3	-	-	-	-	-	-	-	171.0
Sunday	19.6	-	-	-	-	-	-	-	127.4
AV	14.1	0	0	0	0	0	0	0	91.7
SD	6.8	0	0	0	0	0	0	0	44.3
CV	0.5	0	0	0	0	0	0	0	48.4
S-(-)-Mephedrone									
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)								ESTIMATED CONSUMPTION
	Bristol	Oslo	Milan	Utrecht	Castellón	Brussels	Zurich	Copenhagen	UK (Bristol)
Monday	16.1	-	-	-	-	-	-	-	104.7
Tuesday	6.3	-	-	-	-	-	-	-	41.0
Wednesday	8.5	-	-	-	-	-	-	-	55.3
Thursday	7.4	-	-	-	-	-	-	-	48.1
Friday	8.1	-	-	-	-	-	-	-	52.7
Saturday	21.4	-	-	-	-	-	-	-	139.1
Sunday	12.5	-	-	-	-	-	-	-	81.3
AV	11.5	0	0	0	0	0	0	0	74.8
SD	5.6	0	0	0	0	0	0	0	36.1
CV	0.5	0	0	0	0	0	0	0	48.2
(±)-Mephedrone									
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)								ESTIMATED CONSUMPTION
	Bristol	Oslo	Milan	Utrecht	Castellón	Brussels	Zurich	Copenhagen	UK (Bristol)
Monday	31.1	-	-	-	-	-	-	-	202.2
Tuesday	14.9	-	-	-	-	-	-	-	96.9
Wednesday	17.1	-	-	-	-	-	-	-	111.2
Thursday	15.6	-	-	-	-	-	-	-	101.4
Friday	20.8	-	-	-	-	-	-	-	135.2
Saturday	47.7	-	-	-	-	-	-	-	310.1
Sunday	32.1	-	-	-	-	-	-	-	208.7
AV	25.6	0	0	0	0	0	0	0	166.4
SD	12	0	0	0	0	0	0	0	78.3
CV	0.5	0	0	0	0	0	0	0	47.1

S4. Mephedrone

Synthesised in 1929, mephedrone is a stimulant synthetic derivative of cathinone, whose recreational use was documented only in 2007 [26]. Its abuse and associated deaths were reported in several European countries, such as the UK [27] [28]. For these reasons, several modified cathinones were included in the UK Misuse of Drugs Act (class B) in April 2010. EMCDDA (EU Early Warning System) reported that an increased usage of mephedrone was found in the UK in 2014 [29].

Mephedrone is a chiral compound containing one chiral carbon and two enantiomers that differ in potency[30]. Synthetic routes of mephedrone can be via non- and stereoselective methods, even though according to EMCDDA [31], and confirmed by Castrignanò et al. [32], where 'street mephedrone' was found distributed as a racemate, the non-stereoselective route seems preferred ($EF_{illegal\_synth}$=0.5). Also, stereoselective metabolism was found to favour *R*-(+)-enantiomer in pooled human liver microsomes with EFs changing from 0.50±0.00 to 0.57±0.01 throughout 60 minutes of (±)-mephedrone incubation. Stereoselective metabolism was also evidenced in pooled human urine samples (EF_{urine}=0.59±0.05) [32].

S5. Ephedrines

(±)-Norephedrine contains two chiral centres and exists as *1S,2R*-(+)- and *1R,2S*-(-)-norephedrine. Racemic norephedrine is known as phenylpropanolamine. It is used as a decongestant drug [5]. The other two diastereoisomers are *1R,2R*-(-)- and *1S,2S*-(+)-norpseudoephedrine (the latter compound, also known as cathine, is controlled). (±)-Norephedrine can be used as a precursor to produce amphetamine and, less commonly, 4-methylaminorex [5]. Furthermore, an alternative metabolic pathway of amphetamine produces norephedrine, whose content could vary in acid/alkaline urine conditions [19]. Additionally, 5% *1R,2S*-(-)-norephedrine [33] can be produced as derived product of *S*-(+)-amphetamine from methamphetamine metabolism [34].

(±)-Ephedrine contains two chiral centres. The isomers present in nature (e.g. ephedra) are (*1R,2S*)-(-)-ephedrine and (*1S,2S*)-(+)-pseudoephedrine [35]. *1R,2S*-(-)-ephedrine and *1S,2S*-(+)-pseudoephedrine are a bronchodilator and a decongestant respectively, whilst their enantiomers have no medical use. *1R,2S*-(-)-ephedrine and *1S,2S*-(+)-pseudoephedrine are prescription drugs and they are contained also in over-the-counter (OTC) medications. The risk of misuse of nasal decongestants containing such active ingredients brought to a monitoring action of the OTC sale and the scale of methylamphetamine misuse in the UK in 2009 [36] and restrictions on the sale of pseudoephedrine in the USA [37] in order to control the illegal manufacture of methamphetamine.

Table S18 Norephedrine loads.

E1-Norephedrine								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)				ESTIMATED CONSUMPTION (mg/1000 people/day)			
	Bristol	Oslo	Milan	Utrecht	Bristol	Oslo	Milan	Utrecht
Monday	-	25.0	2.7	-	0.0	30.0	3.2	0.0
Tuesday	-	23.1	4.6	--	0.0	27.7	5.5	0.0
Wednesday	-	37.3	3.7	--	0.0	44.8	4.4	0.0
Thursday	-	27.6	3	--	0.0	33.1	3.6	0.0
Friday	-	18.6	2.6	--	0.0	22.3	3.1	0.0
Saturday	-	22.6	4.4	--	0.0	27.1	5.3	0.0
Sunday	-	22.9	2.3	--	0.0	27.5	2.8	0.0
AV	0	25.3	3.3	0	0.0	30.4	4.0	0.0
SD	0	6	0.9	0	0.0	7.1	1.1	0.0
CV	0	0.2	0.3	0	0.0	23.5	27.6	0.0
E2-Norephedrine								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)				ESTIMATED CONSUMPTION (mg/1000 people/day)			
	Bristol	Oslo	Milan	Utrecht	Bristol	Oslo	Milan	Utrecht
Monday	-	30.3	3.8	-	0.0	36.4	4.6	0.0
Tuesday	-	18.3	2.3	-	0.0	22.0	2.8	0.0
Wednesday	7.4	33.9	4	-	8.9	40.7	4.8	0.0
Thursday	2.5	24.4	4.1	-	3.0	29.3	4.9	0.0
Friday	5.1	21	5.1	-	6.6	25.2	6.1	0.0
Saturday	5.3	26.1	4	-	6.9	31.3	4.8	0.0
Sunday	3.6	25.9	3.1	-	4.7	31.1	3.7	0.0
AV	3.4	25.7	3.8	0	4.1	30.8	4.6	0.0
SD	2.8	5.3	0.9	0	3.5	6.3	1.1	0.0
CV	0.8	0.2	0.2	0	84.9	20.5	23.0	0.0
(±)-Norephedrine								
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)				ESTIMATED CONSUMPTION (mg/1000 people/day)			
	Bristol	Oslo	Milan	Utrecht	Bristol	Oslo	Milan	Utrecht
Monday	-	55.2	6.5	-	0.0	66.2	7.8	0.0
Tuesday	-	41.3	6.9	-	0.0	49.6	8.3	0.0
Wednesday	7.4	71.2	7.7	-	8.9	85.4	9.2	0.0
Thursday	2.5	52	7.1	-	3.0	62.4	8.5	0.0
Friday	5.1	39.7	7.7	-	6.6	47.6	9.2	0.0
Saturday	5.3	48.6	8.4	-	6.9	58.3	10.1	0.0
Sunday	3.6	48.7	5.4	-	4.7	58.4	6.5	0.0
AV	3.4	51	7.1	0	4.1	61.2	8.5	0.0
SD	2.8	10.5	1	0	3.5	12.6	1.2	0.0
CV	0.8	0.2	0.1	0	84.9	20.6	13.7	0.0

Table S19 Ephedrine and pseudoephedrine loads and estimates in wastewater.

1R,2S-(-)-Ephedrine						
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)			ESTIMATED CONSUMPTION (mg/1000 people/day)		
	Bristol	Oslo	Milan	Bristol	Oslo	Milan
Monday	-	0.4	0.5	0.0	0.5	0.7
Tuesday	1.8	-	3.8	2.3	0.0	4.9
Wednesday	0.3	-	0.7	0.4	0.0	0.9
Thursday	-	1.6	4.5	0.0	2.1	5.9
Friday	0.4	1.4	-	0.5	1.8	0.0
Saturday	1.2	0	14.3	1.6	0.0	18.6
Sunday	-	1.3	-	0.0	1.7	0.0
AV	0.6	0.7	3.4	0.8	0.9	4.4
SD	0.7	0.7	5.1	0.9	1.0	6.7
CV	1.3	1.1	1.5	117.3	104.6	151.4
1S,2S-(+)-Pseudoephedrine						
	POPULATION-NORMALISED MASS LOADS (mg/1000 people/day)			ESTIMATED CONSUMPTION (mg/1000 people/day)		
	Bristol	Oslo	Milan	Bristol	Oslo	Milan
Monday	126.7	21.9	36.4	139.4	24.1	40.0
Tuesday	81.2	20.9	39.6	89.3	23.0	43.6
Wednesday	98.4	24.1	36.7	108.2	26.5	40.4
Thursday	55.7	20.7	43.5	61.3	22.8	47.9
Friday	134.1	24.8	30.4	147.5	27.3	33.4
Saturday	89.2	18.6	30.4	98.1	20.5	33.4
Sunday	89.4	17.2	33	98.3	18.9	36.3
AV	96.4	21.2	35.7	106.0	23.3	39.3
SD	26.9	2.7	4.8	29.6	3.0	5.3
CV	0.3	0.1	0.1	27.9	12.9	13.6

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